JOURNAL OF AGRICULTURAL RESEARCH

VOL. 47 WASHINGTON, D. C., AUGUST 15, 1933 NO. 4

STUDIES ON PHYSIOLOGIC SPECIALIZATION IN TILLETIA TRITICI AND T. LEVIS IN THE PACIFIC NORTHWEST [1]

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INTRODUCTION

Bunt, or stinking smut, has long been the most important disease of wheat (*Triticum vulgare* Vill.)[3] in the Pacific Northwest. In the United States it is caused by two species of fungi—namely, *Tilletia levis* Kühn, whose spore wall is smooth, and *T. tritici* (Bjerk.) Wint., whose spore wall is reticulated or rough. Despite the development of resistant varieties and of improved methods of seed treatment, losses due to bunt have increased in recent years, not only in the Pacific Northwest but also in the regions of the Middle West [4] where hard red spring and winter wheats are grown. The increased economic importance of bunt has stimulated the study of this disease by research workers in various parts of the country. Their investigations have shown that one of the major contributory factors in the prevalence of bunt has been the appearance of new physiologic forms of the bunt fungi.

The objects of the investigation reported herein were: (1) To determine the prevalence and distribution of the two species of bunt fungi, *Tilletia levis* and *T. tritici*, in the Pacific Northwest; (2) to determine the prevalence and distribution of the physiologic forms of these bunt fungi and the relation between the varieties of wheat and distribution of the physiologic forms; (3) to ascertain whether pathogenicity of the physiologic forms was constant or was easily modified; and (4) to ascertain what correlation, if any, existed between the morphological and cultural characteristics and the pathogenicity of the physiologic forms.

[1] Received for publication Feb. 24, 1933; issued August 1933. Investigations conducted in cooperation with the Washington, Oregon, and Idaho Agricultural Experiment Stations.

[2] The writer is indebted to Dr. A. G. Johnson, principal pathologist, Division of Cereal Crops and Diseases, for helpful suggestions during the course of these investigations and for assistance in the preparation of the manuscript; to Dr. E. F. Gaines, professor of genetics and cerealist, State College of Washington, and agent, Division of Cereal Crops and Diseases, for suggestions regarding field work, for supplying bunt material and wheat varieties, and for aid in the identification of the varieties from which field collections were made; to Dr. F. D. Heald, head of the Division of Plant Pathology, Washington Agricultural Experiment Station, for suggestions and assistance regarding laboratory phases of these investigations; to Dr. E. N. Bressman, associate agronomist, Oregon Agricultural Experiment Station, for supplying bunt material; and to Dr. W. K. Smith, former research assistant, Washington Agricultural Experiment Station, and Dr. C. S. Holton, agent, Division of Cereal Crops and Diseases, for assistance in taking notes.

[3] According to the rules of botanical nomenclature the name of this species is *Triticum aestivum* L.; but as *T. vulgare* is in general use among agronomists and cereal pathologists and geneticists, the writer gives preference to that form.

[4] BOERNER, E. G., HASKELL, R. J., and MEIER, F. C. SMUTTY WHEAT. U.S. Dept. Agr., Rpt. no. 11, 8 p. 1930. [Mimeographed.]

REVIEW OF LITERATURE

Stakman (*17*) [5] has stated that physiologic forms of fungi may be differentiated by (1) pathogenic effects on different species and varieties of host plants, (2) morphological characteristics, (3) cultural characteristics on artificial media, and (4) physicochemical reactions. The economic importance of demonstrating the existence of physiologic specialization of bunt is concerned primarily with pathogenicity. Consequently, most of the investigations consisted of testing the resistance and susceptibility of a number of wheat varieties to bunt collections.

Faris (*3*), in 1924, was the first to present evidence indicating that physiologic specialization occurred in *Tilletia levis* and *T. tritici*. Later, Rodenhiser and Stakman (*14*), Reed (*11*), Roemer (*15*), Gaines (*5*), Weston (*18*), Bressman (*2*), Holton (*7*), Aamodt (*1*), and others demonstrated physiologic specialization of the bunt organisms by testing the pathogenicity of a number of collections to selected varieties.

Although a major portion of the work on physiologic specialization of bunt has consisted of pathogenicity tests, other means of differentiating the forms have been studied. Bressman (*2*) reported that collections had different optimum temperatures for spore germination. He also differentiated one form by the occurrence of masses of bunt spores on the outside of the glumes of the wheat head. Kienholz and Heald (*9*) found that chlamydospore mass cultures of Gaines' [6] forms differed greatly in cultural characteristics on various solid media. They found, however, that these cultural characteristics were not constant. Rodenhiser (*13*) has shown that forms differ in respect to the stunting effect on the culms of the host plants.

MATERIALS AND METHODS

Bunt collections were made from fields in the major wheat-growing sections of Oregon, Washington, and northern Idaho in 1929 and 1930. Where bunt was abundant, as in most fields, the collections were composed of 50 or more bunted heads taken at random from as many plants. In a few fields bunt was so scarce that smaller collections had to suffice. Spores from one or more bunt balls from each head were examined under a microscope in order to determine the proportion of *Tilletia levis* and *T. tritici*. In this manner the heads of each collection were separated according to the species of bunt. Bunt balls from the heads thus separated were ground and used as inoculum for the varietal host testers in order to determine the range of pathogenicity of each collection. Although 60 percent of the collections contained both *T. levis* and *T. tritici*, the occurrence of both species in a single head was rare. Examination of the ground bunt used as inoculum disclosed the fact that in only 7 of the 240 samples were both the rough-spored and smooth-spored species present.

The collections made in 1929 were propagated on Jenkin club spring wheat at Pullman, Wash., in 1930. Unfortunately, part of the soil in the increase plot had been treated with a weed killer and the wheat in that area died. A number of the collections, therefore, were lost. The ranges of pathogenicity of the surviving collections and of collections made in 1930 were determined in 1930–31 by inoculation of differential varieties.

[5] Reference is made by number (italic) to Literature Cited, p. 212.

[6] GAINES, E. F. WHY SMUT HAS BEEN INCREASING. U.S. Dept. Agr., Off. Coop. Ext. Work, Ext. Path. 6(2):14-15. February 1928. [Mimeographed.]

GEOGRAPHIC DISTRIBUTION

Tilletia tritici, the rough-spored species, has long been the predominant one in the Pacific Northwest, and for a long time it was considered to be the only species present there. Kienholz and Heald (*9*) reported that *T. levis*, the smooth-spored species, was found in only 2 of 631 wheat fields examined in 1919 in eastern Washington. However, surveys made by them in 1927 and 1928 showed that *T. levis* was present in all the principal wheat-growing districts of Washington, although *T. tritici* continued to be the predominating species.

The distribution of *Tilletia levis* and *T. tritici* in the Pacific Northwest, as determined from the 1929 and 1930 collections, is shown in

FIGURE 1.—Map showing distribution of *Tilletia levis* and *T. tritici* in Washington, Oregon, and northern Idaho and approximate proportions of each species in field collections made in 1929 and 1930 are indicated as follows:

●= Field collection containing 100 percent *T. tritici* and 0 percent *T. levis*;
▲= Field collection containing 90–99 percent *T. tritici* and 1–10 percent *T. levis*;
■= Field collection containing 50–89 percent *T. tritici* and 11–49 percent *T. levis*;
△= Field collection containing 11–49 percent *T. tritici* and 50–89 percent *T. levis*;
□= Field collection containing 1–10 percent *T. tritici* and 90–99 percent *T. levis*.

figure 1. Although 60 percent of the field collections contained both species, *T. tritici* continued to predominate. This species was found in every field examined, whereas *T. levis* was found in only 60 percent of the fields, and a summation of all the heads examined showed that 81.2 percent were smutted by *T. tritici*. West of the Cascade Mountains, *T. levis* predominated in 8 of the 10 fields examined. East of the Cascades, *T. levis* was most abundant on Albit and Ridit wheats in the extreme eastern part of Washington, on Turkey in both Washington and Oregon, and, regardless of the variety, in fields in the Grande Ronde Valley northeast of La Grande, Oreg., and in the Camas Prairie area southeast of Lewiston, Idaho. In the district extending from

Dayton, Wash., to Heppner, Oreg., very little *T. levis* was found. The wheat varieties chiefly grown there were susceptible to all the known forms of bunt, yet most of the collections contained *T. tritici* alone and none contained more than 10 percent of *T. levis*.

PHYSIOLOGIC FORMS

Previous investigators (*1*, *2*, *5*, *7*, *8*, *11*, *12*, *14*, *15*, *18*) used field collections as the basis for determining physiologic forms of the bunt fungi. Holton (*7*) has pointed out, however, that from what is known of the genetics of the smuts, it is doubtful whether the term "form" can properly be applied to these field collections. The writer (*4*) found that monosporidial lines were haploid and that hybridization readily occurred between the two species, as well as between various so-called "physiologic forms." Although the occurrence of hybridization in nature has not been demonstrated, there appears to be ample opportunity for it to take place in view of the fact that relatively small samples taken at random from fields in the Pacific Northwest showed that 60 percent of the fields contained the two species. The possibility of hybridization is further increased by the presence in this region of several pathogenically distinct strains of bunt (*2*, *6*). The presence of the two species of *Tilletia* in a majority of the fields and the known occurrence of a number of pathogenic strains make it probable that a collection would contain a mixture of forms even if hybridization were not a factor. Furthermore, it must be recognized that nothing is known in regard to the intergrading of the forms and that it is possible to distinguish only those that have very distinct pathogenic properties. Consequently, an attempt to classify collections according to forms can, at best, be but a grouping of those collections possessing similar ranges of pathogenicity, as shown by the percentage of bunted heads produced on each of a limited number of wheat varieties that serve as differential hosts. From the standpoint of simplicity and utility it seems best, for the present at least, to continue to designate those collections having different pathogenic properties as "physiologic forms," although "form groups" probably would be a more accurate designation.

The results of some of the more outstanding pathogenicity tests with collections of *Tilletia levis* and *T. tritici* are shown in table 1.

Although these collections may have contained a mixture of forms, the results were sufficiently distinct to justify an attempt at classification. Forms T-1, T-2, and T-3 possessed pathogenic properties similar to Gaines'[7] forms of the same numbers. Form T-1 was the old *Tilletia tritici* form to which all the differential varieties were resistant, except Hybrid 128, which was susceptible to all forms. Form T-2 was very virulent, attacking Turkey (C.I. 6175), Albit, Hussar, and White Odessa. Form T-3 differed from form T-2 in that it attacked Albit and White Odessa but not Turkey (C.I. 6175) or Hussar. Form T-4 differed from form T-3 in that it attacked Turkey (C.I. 6175) but not Albit. The examples chosen to illustrate forms T-5, T-6, and T-7 make them appear to be distinct. Results from pathogenicity tests of other collections might have been chosen, however, that would have shown a gradual gradation from form T-1 to these three groups. The production of 7.6 and 66.3 percent smut

[7] GAINES, E. F. Op. cit.

in Turkey (C.I. 6175) by forms T-1 and T-5, respectively, undoubtedly justified the separation of these forms. Likewise a separation of forms T-6 and T-7 from form T-5 appeared to be justified by the production of 18.5 percent of smut in Oro by form T-6 and 22.2 percent in Hohenheimer by form T-7. However, as all gradations of infection occurred between these extreme examples, the propriety of setting up an arbitrary percentage number separating the forms is questionable. It is possible that the inoculum obtained from these collections contained a mixture of forms that may be separated by a process of varietal screening. It is also possible that in some varieties there is a complex interrelationship between host resistance and pathogenicity of the fungi and that in these cases it may be impossible to purify a collection for specific host pathogenicity. On the other hand, it appears that some varieties are either resistant or susceptible to certain collections. For example, the susceptibility of Albit to 150 collections of *T. tritici* was determined. Of these collections 133 produced less than 5 percent of bunted heads and 17 produced more than 21 percent of bunted heads.

TABLE 1.—*Physiologic forms of Tilletia tritici and T. levis in representative field collections made in the Pacific Northwest in 1929 and 1930, as shown by results of inoculations of 9 differential varieties of wheat at Pullman, Wash., 1931*

Species	Form no.	Collection no.	Percentage of bunted heads in—								
			Hybrid 128 (C.I.[a] no. 4512)	Turkey (C.I. no. 6175)	Ridit (C.I. no. 6703)	Oro (C.I. no. 8220)	Hohenheimer (C.I. no. 11458)	Turkey selection (C.I. no. 7366)	Albit (C.I. no. 8275)	Hussar (C.I. no. 4843)	White Odessa (C.I. no. 4655)
Tilletia tritici	T-1	A-152	86.8	7.6	0.8	0.4	1.5	1.0	0.5	0	1.6
	T-2	B-108	87.5	60.0	8.7	.2	1.5	0	40.8	28.5	66.7
	T-3	A-155	88.2	4.4	0	0	4.0	1.8	34.8	.2	39.8
	T-4	C-101	90.4	40.9	2.7	0	1.4	0	3.8	0	43.3
	T-5	B-112	84.0	66.3	1.5	.4	1.4	0	.2	0	2.4
	T-6	C-2	39.1	72.0	1.0	18.5	0	2.3	0	0	0
	T-7	C-103	90.5	74.0	.7	2.1	22.2	0	.8	0	1.0
Tilletia levis	L-1	B-107	85.1	87.0	0	3.7	1.4	1.2	0	0	.4
	L-2	A-161	89.1	98.0	8.7	4.5	1.4	1.5	96.2	74.6	90.7
	L-3	C-103	77.1	50.0	26.6	.7	1.5	2.2	1.1	0	1.5
	L-4	A-162	90.0	93.2	.7	20.4	0	3.1	90.3	42.2	70.6
	L-5	A-42	88.1	82.9	1.8	3.0	13.6	.3	6.3	.2	11.3
	L-6	A-107	88.6	15.6	1.9	2.7	3.3	12.8	3.2	0	3.6

[a] C.I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

The inoculation tests with the collections of *Tilletia levis* showed the existence of only two sharply differentiated groups as illustrated by forms L-1 and L-2. Albit, Hussar, and White Odessa were resistant to form L-1 and susceptible to form L-2. Form L-3 differed from form L-1 in that it attacked Ridit. Numerous gradations in percentage of bunt in Ridit were obtained, from 0 percent in the type collection of form L-1 to 26.6 in that of L-3. However, since Weston (*18*) and Bressman (*2*) have shown that Ridit apparently screens out of a collection those forms to which it is susceptible, and since Ridit was very resistant to most of the collections falling in form group L-1, it was arbitrarily decided to place in a new group those collections producing more than 15 percent of bunt in Ridit. The screening effect of Ridit was illustrated by a bunted Ridit plant which was

found in a field of Albit. Inoculum from this plant produced 40.9 percent of bunt in Ridit, while that obtained from Albit plants in the same field produced 11.4 percent of bunt in Ridit. Form L-4 was separated from form L-2 by the virulence of the former to Oro, and the virulence of form L-5 to Hohenheimer separated it from form L-1. Form L-6 was differentiated by the reaction of the two strains of Turkey used in these trials. Both strains were moderately resistant to this form, whereas one (C.I. 6175) was susceptible and the other (C.I. 7366) was very resistant to all the other forms of *T. levis*.

VARIETAL DISTRIBUTION

The relation between the varietal source of the collections and the species and physiologic forms of bunt is shown in table 2.

TABLE 2.—*Relation between the variety of wheat grown and the species Tilletia levis and T. tritici and their physiologic forms contained in field collections made in the Pacific Northwest in 1929 and 1930*

Wheat variety from which collection was made	Number of field collections containing—								
	Only *T. tritici*	*T. tritici* and *T. levis*	*T. tritici* forms—				*T. levis* forms—		
			T-1	T-2	T-3	T-4	L-1	L-2	L-3
Albit	1	13	0	1	9	0	1	9	1
Baart	7	12	10	0	0	0	7	1	0
Pacific Bluestem	1	3	4	0	0	0	3	0	0
Coppei	1	1	1	0	0	0	1	0	0
Dicklow	2	1	3	0	0	0	1	0	0
Federation	14	3	16	0	0	0	3	0	0
Goldcoin (Fortyfold)	3	12	13	1	1	0	8	3	0
Hybrid 123	0	1	0	0	0	0	0	0	0
Hybrid 128	16	11	24	0	0	0	6	0	0
Hybrid 143	0	1	1	0	0	0	0	0	1
Jenkin	7	4	9	0	0	0	3	0	0
Marquis	0	1	1	0	0	0	1	0	0
Mosida	1	3	2	0	2	0	3	0	0
Red Russian	1	2	1	0	0	0	2	0	0
Ridit	0	5	4	0	0	0	1	1	2
Sol (Sun)	1	1	0	0	1	0	0	1	0
Pilcraw	1	0	1	0	0	0	0	0	0
Triplet	11	9	17	1	0	0	8	0	0
Turkey	2	19	17	0	0	0	15	1	1
Mixture and unidentified	4	7	9	0	0	1	5	0	1
Total	73	109	133	3	13	1	68	16	6

Tilletia levis was present in most of the fields of Albit, Ridit, and Turkey varieties resistant to the old form of *T. tritici*. The high percentage of species mixtures in fields of the susceptible Goldcoin (Fortyfold) was due to the fact that this variety was extensively grown in districts where *T. levis* was abundant, particularly in regions west of the Cascades, in the Grande Ronde Valley of Oregon, and in the Camas Prairie region of Idaho. A number of fields of Hybrid 128 and Triplet in these districts also contained both species. *T. tritici* proved to be the prevailing species throughout the wheat-growing section from eastern Washington to north-central Oregon, where bunt-susceptible varieties were the principal ones grown.

It has already been pointed out that a majority of the field collections were mixtures of the two species and that probably many of them also were mixtures of physiologic forms. Consequently, an accurate determination of all the physiologic forms present in each collection could not be made in 1 year. Since the primary object of

the work was to determine the prevalence and distribution of the new physiologic forms, it was decided to dispense with borderline groups and to include in the classification only those forms which were undoubtedly distinct. Therefore those collections that may have contained the borderline forms T-5, T-6, and T-7 of *Tilletia tritici* were grouped with form T-1, and those containing form L-4 and forms L-5 and L-6 of *T. levis* were grouped with forms L-2 and L-1, respectively.

The largest proportion of the collections (84 percent) belonged in the groups containing forms T-1 and L-1. These were the forms of *Tilletia tritici* and *T. levis* to which Ridit, Albit, Hussar, and White Odessa were resistant. Most of the collections placed in these groups attacked Oro, Hohenheimer, and Turkey selection (C.I. 7366) very weakly, while many produced only a small percentage of bunt in Turkey (C.I. 6175).

In the region surveyed in 1929 and 1930, Albit, Ridit, and Turkey (C.I. 6175) were the only resistant wheats grown commercially to any extent. Albit was the best differential variety, and 20 of the 39 collections containing the so-called new forms were obtained from fields of this variety. The one collection of form L-1 from an Albit field was derived from a single bunted head that might have been a volunteer plant of Hybrid 128, a susceptible variety closely resembling Albit. When tested on Ridit, the collections of *Tilletia tritici* were not sufficiently virulent to warrant the establishment of a new form. Three of the four collections of *T. levis* on Ridit were strongly pathogenic. One of these belonged to form L-2, to which Albit, Hussar, and White Odessa were very susceptible and Ridit moderately susceptible. The other two collections bunted Ridit severely but did not attack the other resistant varieties. These collections were classified as form L-3. According to Gaines,[8] Turkey (C.I. 6175) was resistant to the old form of *T. tritici* (form T-1), but was susceptible to the new form T-2 and to all forms of *T. levis*. In the present tests all the *T. tritici* collections obtained on Turkey were similar to Gaines' old form T-1, except that they attacked Turkey (C.I. 6175) normally. Tests with *T. tritici* collections from other varieties gave all gradations of infection on Turkey (C.I. 6175), from cases showing a small percentage, as Gaines found in the old form T-1, to cases in which Turkey was more severely bunted than Hybrid 128. Fifteen of the 17 collections of *T. levis* from Turkey contained forms belonging to group L-1. One collection, from Pomeroy, Wash., bunted Albit severely and was placed in group L-2; and another collection, from southwest of Colfax, Wash., attacked Ridit but not Albit and was placed in group L-3.

Although most of the virulent forms found in this survey were collected from fields of resistant varieties, the exceptions are worthy of note. Form T-2, collection B-108, the most virulent of all the *Tilletia tritici* collections, was obtained at Pendleton, Oreg., from a field of Triplet wheat. The other *T. tritici* collections containing form T-2 were obtained from a field of Albit wheat south of Spokane, Wash., and from a field of Goldcoin (Fortyfold) in the Camas Prairie district southeast of Lewiston, Idaho. Form T-3 was obtained from 9 fields of Albit and 2 fields of Mosida in the vicinity of Pullman, Wash.; and west of the Cascades, from 1 field of Goldcoin (Fortyfold)

[8] GAINES, E. F. Op. cit.

south of Portland, Oreg., and from 1 field of Sol (Sun) wheat north of Kalama, Wash. The one collection of form T-4 was obtained from an unidentified variety on the Camas Prairie of Idaho. Form L-2 of *T. levis* was obtained from 9 fields of Albit near Pullman, Wash.; from 1 field of Baart at Waterville, Wash.; from 3 fields of Goldcoin (Fortyfold) in the Grande Ronde Valley of Oregon; from 1 field of Turkey near Pomeroy, Wash.; and from the field of Sol (Sun) wheat north of Kalama, Wash. (west of the Cascades), in which form T-3 was found. Form L-3 was found only in eastern Washington on Albit, Ridit, and Turkey, and on the Camas Prairie of Idaho on Hybrid 143 and on an unidentified variety, probably selection C. This selection is one of Gaines' varieties that has never been officially released.

SOIL INFESTATION

Soil infestation is a major problem in the wheat-growing sections of the Pacific Northwest, east of the Cascade Range. In the fall of 1929 seed of Hybrid 128, treated with formaldehyde, was sown at Pendleton, Moro, and Corvallis, Oreg., and at Puyallup and Pullman, Wash. Since no bunt was produced at Corvallis or Puyallup, which are west of the Cascades and out of the usual region of soil infestation, it was evident that the seed was clean and that the bunted heads produced at Moro, Pendleton, and Pullman were due to soil infestation. Microscopic examination of the bunted heads and pathogenicity tests showed that only form T-1 of *Tilletia tritici* was present in these collections. Apparently *T. levis* and the more virulent strains of *T. tritici* had not become sufficiently abundant to be important factors in soil infestation.

ADAPTATION AND SCREENING

The question often has been raised as to whether or not the bunt fungi are able to change their virulence on association with resistant varieties so that they will be able to infect such varieties normally. Weston (*18*) and Bressman (*2*) have been able to increase the percentage of bunt in Ridit by inoculating seed of this variety with bunt obtained from bunted Ridit heads. Because of the limited knowledge concerning the genetics of the bunt fungi and the inability to know whether the collections of bunt contain one or more forms, it has been a debatable question as to whether this apparent change in virulence is due to the adaptation of the fungus to the host or to a screening-out process whereby only those forms in the collection are propagated that are able to attack the resistant host normally.

Through the courtesy of Gaines and Bressman, the writer was supplied with their collections. Gaines' form T-2 was his most virulent form of *Tilletia tritici* and had been increased on Hussar for several generations. Hussar was resistant to all other forms of *T. tritici* known to be present in the Pacific Northwest. Bressman's collection no. 28 was his most virulent form of *T. levis*, the only one attacking Oro severely. This collection was obtained in powdered form, and a microscopic examination showed that a small percentage of *T. tritici* was present, as had been noted by Bressman (*2*). This collection was increased on Hybrid 128 and on Oro, at Puyallup, Wash., in the season of 1929–30. Both varieties were severely bunted, but microscopic examinations showed that *T. levis* was the only species of bunt present on Oro, whereas some heads of Hybrid

128 contained *T. tritici* and others contained *T. levis*. However, the two species were not present in any single head. Nine differential varieties were inoculated with *T. levis* from Oro and with *T. tritici* from Hybrid 128, as well as with inoculum of Gaines' form T-2 obtained from six differential varieties. The results are shown in table 3.

TABLE 3.—*Screening effect of host variety on percentages of bunt in the same variety and in certain other varieties in the subsequent crop*

Differential varieties inoculated	C.I. no.	Percentage of bunted heads of differential varieties inoculated with—							
		Bressman's no. 28 of—		Gaines' form T-2 of *Tilletia tritici* from—					
		T. levis from Oro	*T. tritici* from Hybrid 128	Hybrid 128	Ridit	Oro	Hohenheimer	Albit	Hussar
Hybrid 128	4512	75.0	77.5	35.9	38.6	32.7	15.3	68.8	49.4
Turkey	6175	84.2	87.0	45.7	55.6	75.8	68.9	82.2	73.3
Ridit	6703	.5	2.2	.2	.5	.4	0	1.1	1
Oro	8220	58.2	2.2	1.2	(a)	1.6	1.4	2.2	2.6
Hohenheimer	11458	0	13.0	0	(a)	3.0	0	4.8	3.0
Turkey selection	7366	28.2	.4	0	(a)	0	0	.5	0
Albit	8275	0	83.4	26.2	14.9	22.1	7.7	18.5	37.8
Hussar	4843	0	57.2	22.7	24.2	35.3	17.0	27.4	24.3
White Odessa	4655	.5	87.5	46.6	(a)	36.3	16.6	46.6	67.5

a Insufficient inoculum adequately to inoculate all varieties.

There were conspicuous differences in pathogenicity of the *Tilletia levis* and *T. tritici* separated from Bressman's collection no. 28 by varietal screening. Oro was severely bunted by the strain of *T. levis* obtained from this collection, whereas Albit, Hussar, and White Odessa were highly resistant. On the other hand, Oro was resistant to the *T. tritici* obtained from this collection and Albit, Hussar, and White Odessa were susceptible.

The results obtained with Gaines' form T-2 indicated that its pathogenicity had not been changed by adaptation or by the screening effect of the host. A deficiency of smut from Ridit and Hohenheimer probably accounted for some of the variations, but a special effort was made to give each variety its maximum spore load with inoculum derived from that variety. Form T-2 had been propagated on its differential variety (Hussar) for several generations and there was no indication of adaptation or additional screening effect. The pathogenicity of this collection apparently had been purified to such an extent that it was not altered by being propagated on the other differential hosts used.

MORPHOLOGICAL CHARACTERS

During the examination of the spores to determine the species present in the collections, a number of heads were found which contained spores having distinct morphological characteristics. These spores differed in size, color, and character of reticulations. Some of the bunt balls were very hard and it was exceedingly difficult to powder them, even with a mortar and pestle, while other bunt balls were so soft that it was virtually impossible to shell them from the

head without rupturing the wall of the spore ball and allowing the enclosed spores to run out. Bunted heads containing these morphological types were separated from the collection proper and propagated on Jenkin wheat at Pullman, Wash., in the summer of 1930. An examination of the increased material showed that the distinguishing morphological characters had been retained.

The standard varietal host testers were inoculated with the increased material the following season, but no correlation was found between morphological characters and pathogenicity, with the possible exception that the selections having large spores with very pronounced reticulations produced little bunt even on susceptible Hybrid 128. The morphological characteristics of spores and spore balls were transferred to the succeeding generation, but the pathogenicity tests showed that all these variable selections belonged to form T-1.

DIFFERENTIAL EFFECT OF FIELD TEMPERATURES

The effect of temperature on percentage of bunt produced by five of Gaines'[9] physiologic forms was tested by sowing inoculated seed of a susceptible variety (Hybrid 128) and that of a resistant variety (Albit) in quadruplicate plots at weekly intervals from September 12 to November 11, 1930. The temperature during this series of sowings ranged from an average of 66° F. in the first week, which was too high for satisfactory bunt infection, to 35° in the last week, which was too low. This wide variation in temperature, however, produced no significant differences in the percentages of bunt among the forms tested.

CULTURAL STUDIES

Cultural studies are an important phase in an investigation of physiologic forms, not only for finding new forms but also because of the possibility of establishing a correlation between cultural and pathogenic characteristics. If it were possible to determine the pathogenicity of a bunt collection by its cultural characteristics a great deal of time would be saved, because cultural characteristics may be determined in the laboratory in a few weeks, whereas field pathogenicity tests require from one to several years.

The cultures used in these studies were obtained from Gaines'[10] physiologic forms T-1, T-2, T-3, L-4, L-5, L-6, and L-7. The writer has continued to apply the terms T-1, T-2, and T-3 to collections having pathogenic properties similar to those of Gaines' three physiologic forms of *Tilletia tritici*. Because of the recent extensive investigations of physiologic specialization in the bunt fungi, it appeared desirable to start a system of physiologic-form classification wherein forms of the two species would be completely separated. Consequently, those collections having the pathogenic properties of Gaines' form L-5 have been placed in the L-1 group. Pathogenicity tests showed that while Gaines' forms L-4, L-6, and L-7 may have possessed minor pathogenic differences, they were sufficiently alike to be placed in the L-2 group. Hence, in these cultural studies, the cultures derived from Gaines' forms L-4, L-6, and L-7 have been designated L-2-a, L-2-b, and L-2-c, respectively.

[9] GAINES, E. F. Op. cit.
[10] GAINES, E. F. Op. cit.

The method whereby single primary sporidial cultures of bunt were obtained has already been described (*4*). A number of media were tested in addition to those used by Sartoris (*16*) and Kienholz and Heald (*9*) in their studies on culturing the bunt fungi, but none was found to be superior to potato-dextrose agar, either for mycelial growth or for differentiating properties.

Types of Mycelium in Cultures

Preliminary tests showed that the two distinct types of mycelial growth, with numerous variations, observed by Sartoris (*16*) and Kienholz and Heald (*9*) in chlamydospore mass cultures, were produced also by cultures derived from single sporidia. Microscopic examination revealed that the white cartilaginous heaped-up type was composed largely of fine mycelial threads and produced very few secondary sporidia in the early stages of formation and none later. The few sporidia produced by the white type of mycelium were found to reproduce only this type of mycelial growth. The gray flat type of mycelial growth was composed largely of mycelial threads that were much branched and twisted and were 3 to 4 times as thick as those of the white type. The gray mycelium produced an abundance of secondary sporidia from which the gray type of mycelium developed in the early stages of growth. Later the white type of growth was also produced in these cultures.

Effect of Temperature on Mycelial Growth

The effect of temperature on mycelial growth was determined by growing monosporidial cultures of Gaines' [11] physiologic forms of bunt on potato agar with 2 percent dextrose in incubators at 7°, 12°, and 18° C., and at room temperature (20° to 25°). In general, cultures of all the forms responded similarly. At 7° growth was slow, and only the gray type of mycelium was produced; no chromogenesis of the medium occurred. At 12° and 18° growth was fairly rapid and both the gray and white types of mycelium were produced. At the lower temperature the gray type predominated, whereas at the higher temperature the two types were produced in about equal quantities, although considerable variation occurred between the individual cultures. At room temperature (20° to 25°) mycelial growth was slow and almost entirely of the white type. Chromogenesis was prominent at temperatures from 12° to 25°, except in cultures of form L-1.

Effect of Sugar Content of the Medium

Monosporidial cultures of six-physiologic forms of bunt were grown on potato agar containing 0, 0.5, 1.0, 2.0, 4.0, and 6.0 percent of dextrose, respectively, and incubated at 15° to 18° C. It was found that these percentages of sugar in the medium produced a gradation from the gray to the white type of mycelium similar to that produced by different temperatures. Although the individual cultures differed considerably in appearance, they showed the same general tendency in regard to chromogenesis and type of mycelium produced. The effect of the sugar (dextrose) content of the medium on chromogenesis and mycelial development of a monosporidial culture of

[11] Gaines, E. F. Op. cit.

Tilletia levis form L-2-b is shown in figure 2. Only the gray type of mycelium and no chromogenesis was produced on the plain potato agar. There was a tendency to produce the white type of mycelium at the

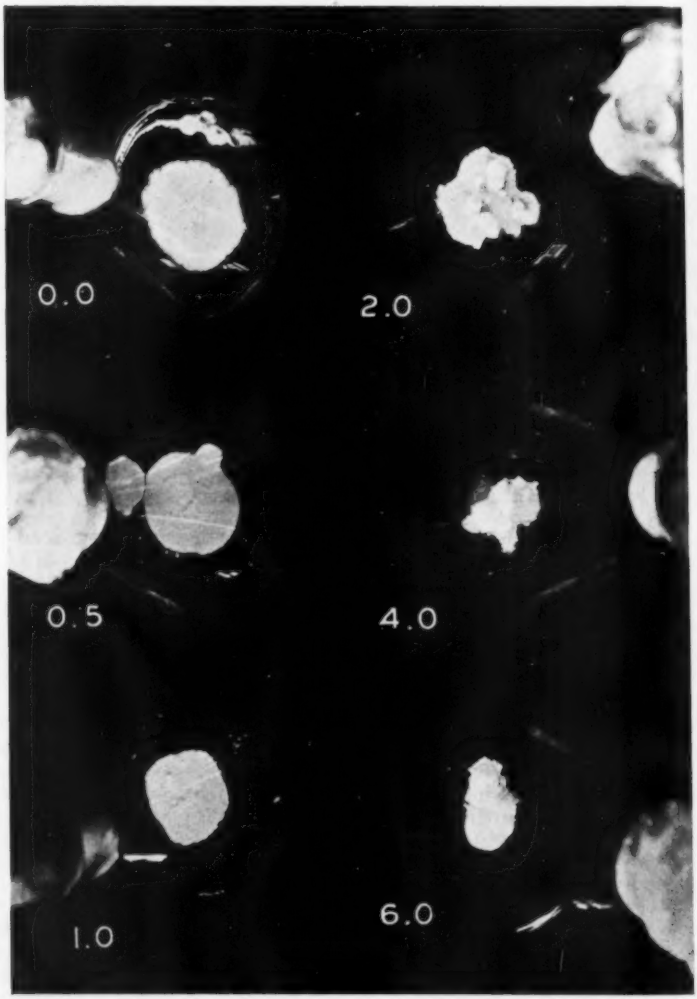

FIGURE 2.—Cultures derived from a single primary sporidium of *Tilletia levis* form L-2-b grown on potato agar containing 0, 0.5, 1.0, 2.0, 4.0, and 6.0 percent of dextrose, as indicated, and incubated at 15° to 18° C. The proportion of the white heaped-up mycelium to that of the gray flat type increased with the sugar content of the medium.

center of the colony grown on potato agar with 0.5 percent of dextrose. With each increase in the percentage of dextrose in the agar, there was an increase in the proportion of the white to the gray type of mycelium. The mycelium produced on the potato agar with 6 percent of dextrose

was entirely white except for a few marginal spots of gray. Cultures of Gaines' forms T-1, T-2, and T-3 of *T. tritici* and L-2-a and L-2-b of *T. levis* produced chromogenesis in all media to which dextrose had been

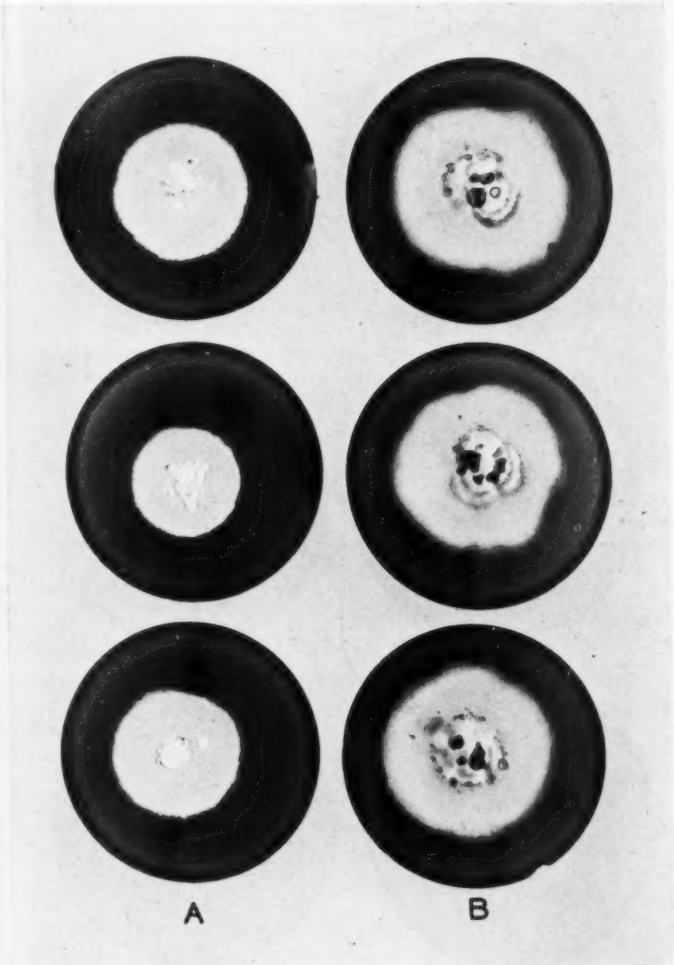

FIGURE 3.—Cultures, in triplicate, showing the uniformity of the gray (A) and the white (B) types of colonies derived from a monosporidial isolation of *Tilletia tritici* form T-3 (culture 263) and grown on potato agar containing 1 percent of dextrose, at 16° C.

added. Form L-1 of *T. levis* did not produce chromogenesis under any condition.

COMPARISON OF CULTURAL CHARACTERISTICS

The characteristics of 34 monosporidial cultures of seven physiologic forms of *Tilletia tritici* and *T. levis* were studied by growing them in triplicate at 16° C. in flasks of potato agar containing 1 percent of

dextrose. Figures 3 and 4 show that a fair degree of uniformity of cultural characteristics was obtained when care was exercised to control conditions and to select the inoculum. Figure 3 shows triplicate cultures of monosporidial culture 263 of *T. tritici* form T-3. The colonies

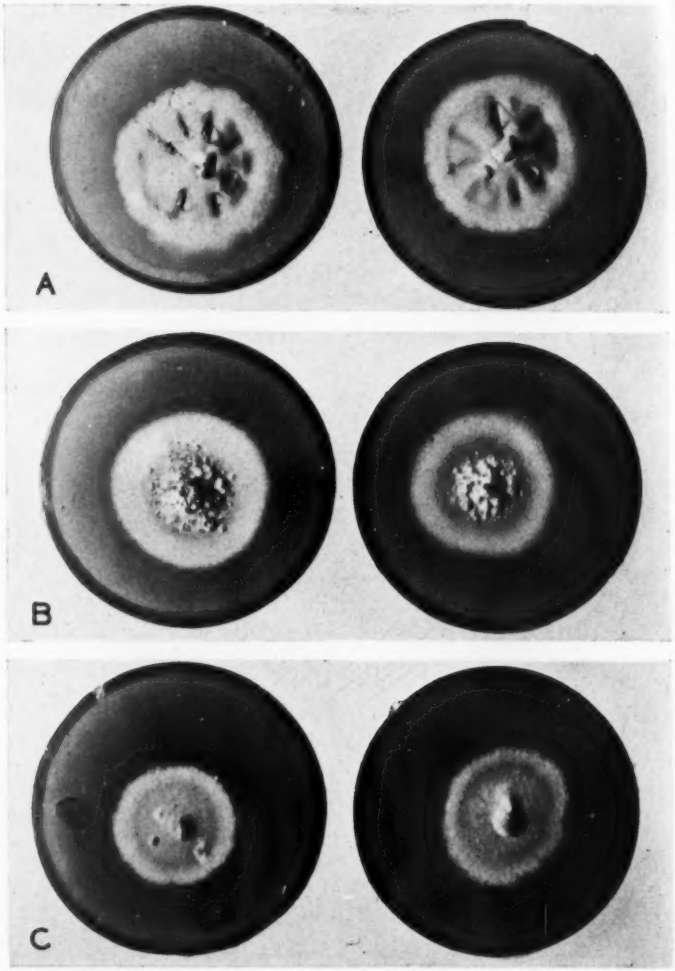

FIGURE 4.—Duplicate cultures (side by side) of three monosporidial isolations, (A) no. 225, (B) no. 284, and (C) no. 287, of *Tilletia levis* form L-2-c, grown on potato agar containing 1 percent of dextrose, at 16° C. The uniformity of cultures of the same monosporidial isolation and the variability of cultures of monosporidial isolations from the same pathogenic form are shown.

produced from the gray mycelial inoculum are shown at the left (A). The mycelial inoculum for those at right (B) was obtained from raised white growths similar to those seen in the center of the gray colonies at left (A); that is, a culture derived from a single primary sporidium of

T. tritici produced colonies possessing distinctly different cultural characteristics. In figure 4 are shown in duplicate (side by side) cultures of three monosporidial isolations of *T. levis* form L-2-c derived from the same wheat head. Care was taken to secure the gray type of inoculum in each instance, and the uniformity of the inoculum was shown by the similarity of the two duplicate cultures in each case. The differences in the tendency of the individual cultures to produce the white and gray types of mycelium were marked. Culture 287 consisted almost entirely of the gray type, whereas culture 225 had changed to the white type, and culture 284 was composed of about equal parts of both gray and white. Although these cultures were obtained from the same physiologic form there was but little resemblance between them. As a matter of fact, culture 287 of form L-2-c of *T. levis* resembled the gray mycelial colony of culture 263 of form T-3 of *T. tritici* more closely than it did the other cultures of the physiologic form to which it belonged.

A study was made of the 12 monosporidial cultures of *Tilletia tritici* form T-3, whose paired pathogenicity had been determined (*4*). Six of these cultures belonged to sex group B (fig. 5) but showed little uniformity in cultural characteristics. Cultures 263 and 240 resembled one another somewhat, as did cultures 303, 304, and 306, but culture 243 was distinctly different from any of the others. Figure 6 shows the cultures of sex groups A, C, and D of form T-3. Cultures 213 and 209 of sex group A were fairly similar and resembled cultures 240 and 263 of sex group B, whereas cultures 302 and 305 of sex group D resembled one another and also resembled cultures 303, 304, and 306 of sex group B. Culture 235 of sex group C resembled somewhat cultures 302, 303, 304, 305, and 306 of sex groups A and B. Culture 262, which either belonged to another sex group or was sterile, differed culturally from all of the other cultures.

The monosporidial cultures of *Tilletia tritici* forms T-1 and T-2 and *T. levis* forms L-1, L-2-a, and L-2-b were as variable as those of *T. tritici* form T-3 and *T. levis* form L-2-c, with the single exception that cultures of *T. levis* form L-1 were not chromogenic. A preliminary study was made of the cultural characteristics of chlamydospore cultures of a number of collections in conjunction with field pathogenicity tests. No cultural characteristics were found that would aid in the identification of pathogenic forms, except that cultures of *T. levis* that were not chromogenic invariably belonged to the same pathogenic group as did *T. levis* form L-1. However, cultures of some collections that fell in the *T. levis* L-1 pathogenic group were chromogenic.

DISCUSSION

The problem of physiologic specialization of *Tilletia tritici* and *T. levis* is complicated by the limited knowledge of the genetics of these fungi. Rodenhiser (*12*) has shown the existence of numerous physiologic forms of a number of other cereal smuts by differences in cultural characteristics. However, since the mycelial stage of the bunt fungi is haploid (*4*) and the pathogenic and propagative phase is diploid (*10*), it is doubtful whether cultural characteristics should be considered the basis for the determination of physiologic forms.

The present studies have shown that different monosporidial cultures (haploid) derived from a single bunted head might be distinctly

different, despite the fact that the inoculum had been subjected to the purifying effects of varietal screening for several generations. As a

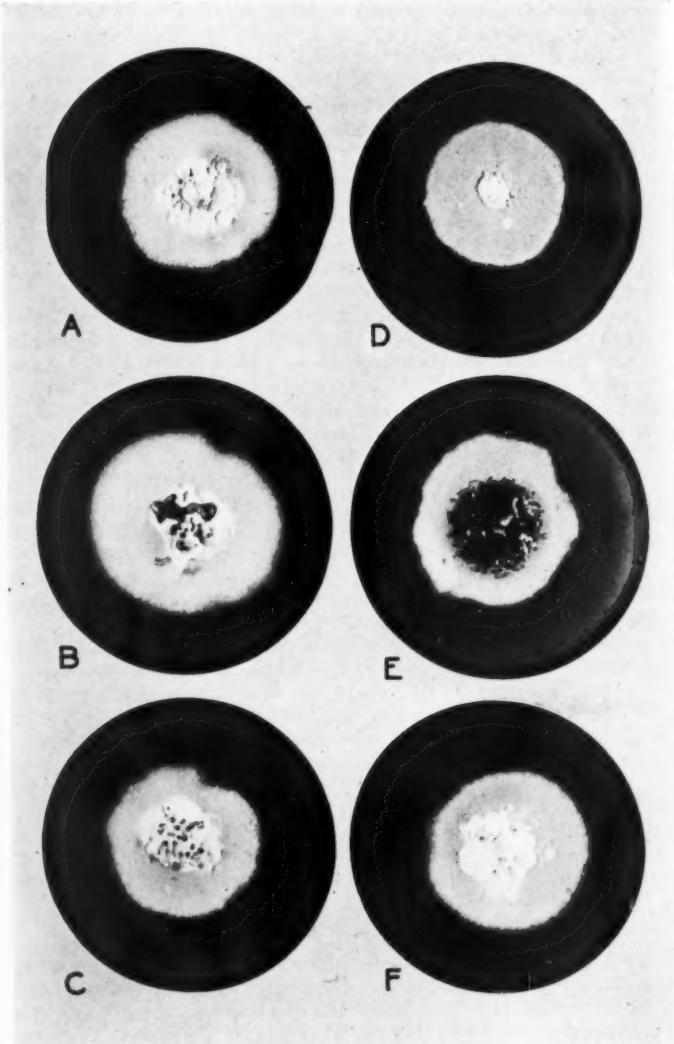

FIGURE 5.—Six monosporidial cultures, (A) no. 306, (B) no. 304, (C) no. 303, (D) no. 263, (E) no. 243, and (F) no. 249, of *Tilletia tritici* form T-3, sex group B, grown on potato agar containing 1 percent of dextrose at 16° C., showing the lack of uniformity in cultural characteristics.

matter of fact, each one of the 12 cultures of a single pathogenic form might have been regarded as a distinct physiologic form on the basis of differences in cultural characteristics. In some cases a closer resem-

blance was found between the cultural characteristics of monosporidial cultures from the two species of *Tilletia* on wheat than between cultures derived from a single pathogenic form of either species. The

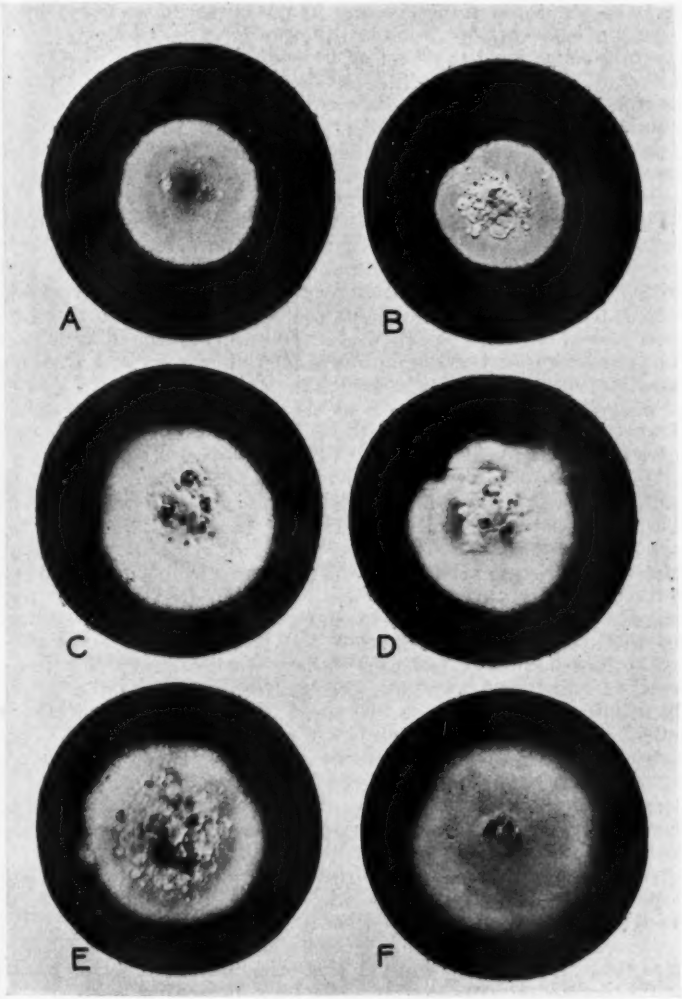

FIGURE 6.—Six monosporidial cultures, (A) no. 209, (B) no. 213, (C) no. 302, (D) no. 305, (E) no. 235, and (F) no. 262, of *Tilletia tritici* form T-3 grown on potato agar containing 1 percent of dextrose, at 16°C., showing the different cultural characteristics of monosporidial isolations. Cultures 209 and 213 belonged to sex group A, nos. 302 and 305 to sex group D, no. 235 to sex group C, and no. 262 either was sterile or belonged to another group.

existence of great differences between the cultural characteristics of monosporidial cultures when derived from a single pathogenic form confirms the opinion that hybridization is the rule in the bunt fungi

and that a study of cultural characteristics with a view to determining pathogenicity is of little value, at least until pathogenic forms are based on something more homogeneous than field collections.

Up to the present time field collections of chlamydospores have been the basis for determining the physiologic forms of bunt. Usually if a series of more or less resistant differential varieties inoculated with a certain collection has produced percentages of infection sufficiently different from those produced by inoculation with other collections, the collection tested has been termed a new form. The only attempt at purifying the forms in a collection has been through a limited use of the "varietal screening" method. By this method the inoculum is increased on a resistant variety, and any included forms that are unable to attack this resistant variety are screened out.

The possibilities of the "varietal screening" method of form purification have just been touched upon, yet some significant results have been obtained. Bressman (*2*) and Weston (*18*) have shown that in some cases the original collection produced only a small percentage of bunt in Ridit, but when this bunt was again returned to this variety a strain was obtained which was capable of bunting Ridit severely. The value of varietal screening was further illustrated with Bressman's collection no. 28. Although he found a slight mixture of species in the collection, he reports it (*2*) as a form of *Tilletia levis* capable of infecting Oro severely and Albit and Hussar moderately. When this collection was increased on Oro, all the *T. tritici* was screened out, and the *T. levis* caused severe infection of Oro but was incapable of attacking Albit or Hussar. On the other hand, Oro was resistant to the *T. tritici* inoculum produced on Hybrid 128, but Albit and Hussar were severely bunted. Thus two strains of bunt having distinctly different pathogenic properties were separated from a single collection by the use of two varieties of wheat. It is possible that other pathogenically distinct strains might have been separated if additional varieties had been used as "screens." The purifying effect of increasing the inoculum on a resistant variety was illustrated by *T. tritici* form T-2, which had been increased on Hussar for a number of years. When inoculum of this form, obtained from Hybrid 128, Ridit, Oro, Hohenheimer, Albit, and Hussar, was again applied to these varieties there were no significant differences in pathogenicity. The propagation of this collection on its differential variety had purified it to such an extent that its pathogenicity was not altered by the screening effect of any of the resistant varieties on which it was subsequently propagated.

In the present study both *Tilletia levis* and *T. tritici* were found in 60 percent of the fields from which bunt collections were made throughout the wheat-growing areas of the Pacific Northwest. When it is considered that these collections consisted of from 50 to 100 heads taken from a very limited area of each field, it seems probable that both species of bunt may have been present in even a greater percentage of the fields. Besides the two species, apparently there are an indefinite number of physiologic forms in the Pacific Northwest area. The separation of the forms by varietal screening appears promising, but it will require a number of years to determine the value as well as the limitations of this method.

The problem of determining the number of physiologic forms in a collection is complicated not only by the possibility of physical

mixtures of forms, but also by the possibility of hybridization. It has been shown (*4*) that hybridization occurs between the two species *Tilletia levis* and *T. tritici*, as well as between the various forms within each species. The importance of hybridization under natural conditions is not known, but observational evidence (*9*) seems to indicate that it does occur.

The bunt survey of 1929 and 1930 showed that although *Tilletia tritici* continued to be the predominant species in the Pacific Northwest, *T. levis* was widely distributed and was present in all the major wheat-growing areas of that region. Pathogenicity tests of the collections showed that the less virulent strains of both species were most abundant. As a general rule, the less virulent form T-1 of *T. tritici* was found on bunt-susceptible varieties. When *T. levis* was obtained from susceptible varieties, it likewise usually proved to be the less virulent form L-1. As was to be expected, collections made from varieties resistant to the old forms usually contained the so-called "new," more virulent forms. The new forms were especially abundant in districts where the resistant varieties Albit and Ridit were grown. They were also prevalent on susceptible as well as on resistant varieties on the Camas Prairie of Idaho and in the Grande Ronde Valley of Oregon, two districts considerably separated from the main wheat-growing area. In a few instances the new virulent forms were obtained from susceptible varieties in widely scattered localities in the main wheat-growing region. This indicated that the new forms had become established and would probably become prevalent over the entire region in a few years, unless methods of combating their spread were applied.

A number of means are available for retarding the dissemination of these new physiologic forms, but their application requires the intelligent cooperation of all wheat growers in the regions concerned. In the first place, locally grown seed should be used wherever possible; and, secondly, all seed, whether locally grown or imported, should be efficiently treated, as has been recommended by Heald and Gaines (*6*) and Holton (*8*). This applies to seed of resistant varieties as well as to that of susceptible varieties, since the destruction of seed-borne spores would obviate to a considerable extent the screening effect of resistant varieties whereby forms of bunt capable of infecting them are propagated. A third possibility is the intelligent use of a rotation of resistant wheat varieties. For example, at the present time no form capable of attacking both Turkey selection (C.I. 7366) and Albit is known. If Albit should become bunted in one locality, a concerted change, for 1 or 2 years, to Turkey selection (C.I. 7366) or to a variety possessing similar resistance should eliminate those forms capable of attacking Albit.

It has been shown that forms of bunt capable of infecting Albit and Ridit are rather prevalent in fields of these varieties. Except in a few instances, these forms are not sufficiently abundant to cause serious economic losses at present (1931), and consequently seed treatment often is not practiced. Unless an adequate seed-treatment program is adopted it seems probable that it will be only a few years until the more virulent forms will become abundant enough to cause serious losses to these resistant varieties and perhaps to others that may be developed in the future.

SUMMARY

Bunt collections were made from 182 fields in the principal wheat-producing areas of Oregon, Washington, and northern Idaho.

Tilletia tritici was found in every collection, whereas *T. levis* was present in only 60 percent of the collections. Of the two species, *T. levis* was the more abundant west of the Cascade Mountains and was present in all the wheat-growing regions, although *T. tritici* was the prevailing species east of the Cascades.

Pathogenicity tests of the collections indicated that at least 7 forms of *Tilletia tritici* and 6 forms of *T. levis* were present. In some collections there appeared to be an intergrading of one form with another, indicating form mixtures or hybridization, whereas other collections had sharply differentiated pathogenic properties.

Eighty-four percent of the collections contained only the less virulent forms of *Tilletia levis* or *T. tritici*, forms unable to infect resistant varieties such as Albit, Ridit, Hussar, and White Odessa.

Although most of the new virulent forms were obtained chiefly from fields of Albit and Ridit, they were also found in a few collections from susceptible varieties in widely scattered localities.

The presence of the new virulent forms of both *Tilletia levis* and *T. tritici* on susceptible as well as on resistant varieties makes the adoption of cultural practices imperative in order to impede the spread of these more virulent forms.

Pathogenically distinct forms were readily separated by the screening effect of resistant varieties. However, varietal screening had no effect on the pathogenicity of a collection propagated on a resistant variety, such as Hussar, for several years.

Morphological variations such as size, color, and character of the reticulations of the spore wall, as well as hardness of the bunt ball, were found to be heritable, but no definite correlation was established between these characteristics and pathogenicity.

Variation of the temperature during the period of infection did not cause significant differences in the percentage of bunt produced by five pathogenically distinct physiologic forms.

The characteristics of monosporidial cultures of *Tilletia levis* and *T. tritici* were exceedingly variable. In some instances monosporidial cultures of the two species resembled one another more closely than did those from the same pathogenic form.

No correlation was obtained between cultural characteristics and pathogenicity, except that nonchromogenic cultures of *Tilletia levis* invariably belonged to the less virulent pathogenic form that did not attack Albit, Hussar, or White Odessa.

LITERATURE CITED

(1) Aamodt, O. S.
1931. varietal trials, physiologic specialization, and breeding spring wheats for resistance to tilletia tritici and t. levis. Canad. Jour. Research 5 : 501–528, illus.

(2) Bressman, E. N.
1931. varietal resistance, physiologic specialization, and inheritance studies in bunt of wheat. Oreg. Agr. Expt. Sta. Bul. 281, 44 p., illus.

(3) Faris, J. A.
1924. factors influencing the infection of wheat by tilletia tritici and t. levis. Mycologia 16 : 259–282, illus.

(4) FLOR, H. H.
1932. HETEROTHALLISM AND HYBRIDIZATION IN TILLETIA TRITICI AND T. LEVIS. Jour. Agr. Research 44 : 49–58.
(5) GAINES, E. F.
1928. NEW PHYSIOLOGICAL FORMS OF TILLETIA LEVIS AND T. TRITICI. Phytopathology 18 : 579–588.
(6) HEALD, F. D., and GAINES, E. F.
1930. THE CONTROL OF BUNT OR STINKING SMUT OF WHEAT. Wash. Agr. Expt. Sta. Bul. 241, 30 p., illus.
(7) HOLTON, C. S.
1930. A PROBABLE EXPLANATION OF RECENT EPIDEMICS OF BUNT IN DURUM WHEATS. Phytopathology 20 : 353–357, illus.
(8) ———
1931. THE RELATION OF PHYSIOLOGIC SPECIALIZATION IN TILLETIA TO RECENT EPIPHYTOTICS OF BUNT IN DURUM AND MARQUIS WHEATS. Phytopathology 21 : 687–694, illus.
(9) KIENHOLZ, J. R., and HEALD, F. D.
1930. CULTURES AND STRAINS OF THE STINKING SMUT OF WHEAT. Phytopathology 20 : 495–512, illus.
(10) RAWITSCHER, F.
1914. ZUR SEXUALITÄT DER BRANDPILZE: TILLETIA TRITICI. Ber. Deut. Bot. Gesell. 32 : 310–314, illus.
(11) REED, G. M.
1927. PHYSIOLOGIC RACES OF BUNT OF WHEAT. Amer. Jour. Bot. 15 : 157–170.
(12) RODENHISER, H. A.
1928. PHYSIOLOGICS SPECIALIZATION IN SOME CEREAL SMUTS. Phytopathology 18 : 955–1003, illus.
(13) ———
1931. STUNTING OF WHEAT CAUSED BY TILLETIA LEVIS AND T. TRITICI. Jour. Agr. Research 43 : 465–468.
(14) ——— and STAKMAN, E. C.
1927. PHYSIOLOGIC SPECIALIZATION IN TILLETIA LEVIS AND TILLETIA TRITICI. Phytopathology 17 : 247–253, illus.
(15) ROEMER, T.
1928. GIBT ES BIOLOGISCHE TYPEN VON STEINBRAND (TILLETIA TRITICI) DES WEIZENS? Kühn Arch. 19 : 1–11.
(16) SARTORIS, G. B.
1924. STUDIES IN THE LIFE HISTORY AND PHYSIOLOGY OF CERTAIN SMUTS. Amer. Jour. Bot. 11 : 617–647, illus.
(17) STAKMAN, E. C.
1928. RACIAL SPECIALIZATION IN PLANT DISEASE FUNGI. *In* Lectures on Plant Pathology and Physiology in Relation to Man. . . . Mayo Foundation Lectures 1926–1927, p. 93–150, illus. Philadelphia and London.
(18) WESTON, W. A. R. DILLON.
1929. RESISTANCE OF WHEAT VARIETIES TO BUNT (TILLETIA CARIES) Nature [London] 123 : 243.

A MORPHOLOGICAL STUDY OF FLOWER AND SEED DEVELOPMENT IN CABBAGE[1]

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INTRODUCTION

The solution of many of the problems associated with cabbage-seed growing and commercial cabbage production lies in the field of plant breeding. Few of the vegetable crop plants are so variable as the cabbage family—the Cruciferae. Most of the common varieties of cabbage (*Brassica oleracea capitata* L.) are lacking in uniformity. There is an urgent demand on the part of both seedsmen and commercial cabbage growers for a standardization of varieties, so that a variety will represent a definite type, rather than a mixture of various types as is now so often the case.

It has been demonstrated that at least some of the cabbage diseases can be controlled by the use of strains selected for their resistance to these diseases. Most of the common cabbage varieties can no doubt be greatly improved by scientific methods of breeding and selection.

Any attempt to solve these problems through breeding methods requires a knowledge of the morphology of the cabbage flower. The method of pollination, the time required for fertilization, and a general knowledge of the flower structure and its development are important in a breeding program. It was for the purpose of answering some of the questions relating to the cabbage flower and its development that this study was undertaken.

MATERIAL AND METHODS

The first material was collected during the winter of 1929 and the spring of 1930. The plants were grown from seed of the Early Jersey Wakefield variety planted September 4, 1929. All material used in these studies was grown at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D.C. When the plants were about 3 inches high they were transplanted to the field, where they were overwintered. Material for study was collected at intervals of 2 weeks from November 15 to April 25.

A second lot of plants of the same variety was started from seed on August 25, 1930. The plants were set in the field and overwintered as in 1929. Material was collected at intervals of 2 weeks from the middle of November to the latter part of May.

Material was also collected during the fall and winter of 1930 and 1931 from mature plants which were set in the field in the summer of 1930.

The tissues were placed immediately in a killing fluid. Several such agents, including Bouin's, Carnoy's, medium chromoacetic acid, and formalin acetic alcohol were used in the early part of the work. It was later found necessary to use a killing agent in which the tissues could be kept for some time without injury, as it was not convenient to carry the materials through the dehydrating, clearing, and embedding processes immediately after they were collected. The formalin acetic alcohol solution, made up from 50 cc of 95 percent

[1] Received for publication Mar. 9, 1933; issued August 1933.

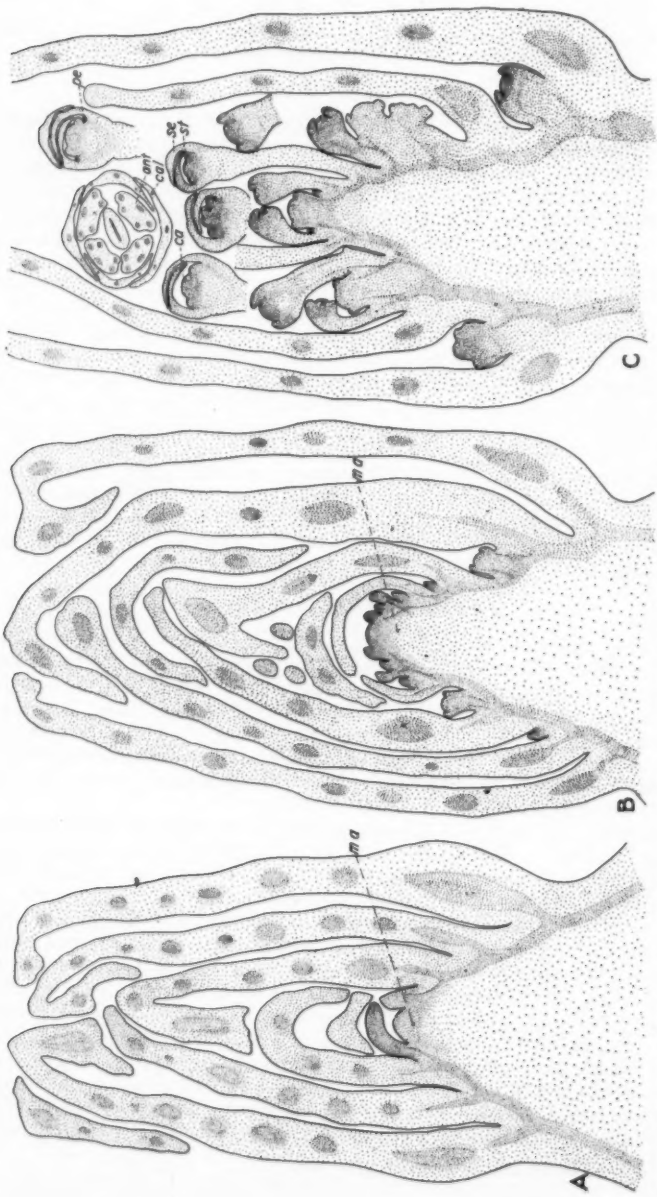

FIGURE 1.—Main axis of cabbage stem: A, Before differentiation of flower parts; B, in early stage of flower differentiation; C, in late stage of flower development. × 16.
(For explanation of abbreviations see opposite page.)

alcohol, 5 cc glacial acetic acid, 5 cc commercial formalin, and enough distilled water to bring the total volume up to 100 cc, was found to be satisfactory for this purpose. Flemming's weaker osmic acid fixing agent was employed for anthers in which division of the pollen mother cells was to be studied.

The waxy epidermis of *Brassica* is very resistant to penetration of the killing agent. Air bubbles under the bracts of the flower also interfered with the killing process. The difficulty in securing rapid penetration and killing was overcome by subjecting the material to a condition of partial vacuum immediately after placing it in the solution.

After dehydrating in a series of alcohols of varying concentration, including absolute alcohol, the materials were cleared in xylol and embedded in paraffin having a melting point of about 55° C.

Delafield's haematoxylin was found to be the most satisfactory stain for most of the structures studied. Heidenhain's iron-alum haematoxylin was used for embryo sacs and for the various stages of pollen development.

DIFFERENTIATION OF FLOWER PRIMORDIA

The time of differentiation of flower primordia in cabbage has been found to depend very largely on temperature relations. Miller [2] has shown that the cabbage plant is capable of continuous vegetative growth without seed formation, at least for several years, if the plant is never subjected to low temperature. Boswell [3] found that the differentiation of flower primordia in young cabbage plants is closely correlated with the size of the plant at the time it is exposed to low temperature. Low temperature failed to induce flower development unless the plant had reached a certain size as indicated by a stem diameter of about 6 mm.

Many sections were prepared from material collected from the plants started from seed on September 4, 1929, and August 25, 1930. The first indication of differentiation of flower primordia in the material started September 4, 1929, was found in terminal buds killed March 3. In the material started from seed on August 25, 1930, the first differentiation was found in material killed February 23. This confirms the findings of Boswell,[3] that differentiation in immature cabbage plants overwintered in the open takes place in early spring.

[2] MILLER, J. C. A STUDY OF SOME FACTORS AFFECTING SEED-STOCK DEVELOPMENT IN CABBAGE. N.Y. (Cornell) Agr. Expt. Sta. Bul. 488, 46 p., illus. 1929.

[3] BOSWELL, V. R. STUDIES OF PREMATURE FLOWER FORMATION IN WINTERED-OVER CABBAGE. Md. Agr. Expt. Sta. Bul. 313, p. 69–145, illus. 1929.

EXPLANATION OF ABBREVIATIONS IN ILLUSTRATIONS

a c, Archesporial cell
an, Antipodals
ant, Anther
ca, Carpels
cal, Calyx
cot, Cotyledons
cyt, Cytoplasm
der, Dermatogen
d m, Disintegrating megaspores
egg nu, Egg nucleus
emb, Embryo
emb c, Embryo cell
emb f, Embryo filament
en, Endothecium
end, Endosperm
ep, Epidermis
epi, Epicotyl
e s, Embryo sac
fu, Funiculus
hyp, Hypocotyl
hypo, Hypophysis
i i, Inner integument
m, Megaspore
m a, Main axis
m l, Middle layer
m m c, Megaspore mother cell
mi, Micropyle
nu, Nucellus
o, Ovary
o i, Outer integument
ov, Ovule
o w, Ovary wall
p nu, Polar nuclei
pe, Petals
peri, Periblem
pig la, Pigmented layer
pl, Placenta
ple, Plerome
p m c, Pollen mother cells
p o, Points of exit
pol, Pollen
pol tu, Pollen tube
r c, Rootcap
se, Sepals
sp, Sperms
st, Stamens
st l, Stigmatic lobe
sty, Style
sup la, Supporting layer
sus, Suspensor
syn, Synergids
tu, Tapetum
te, Tetrads
vac, Vacuole
v n, Vegetative nucleus

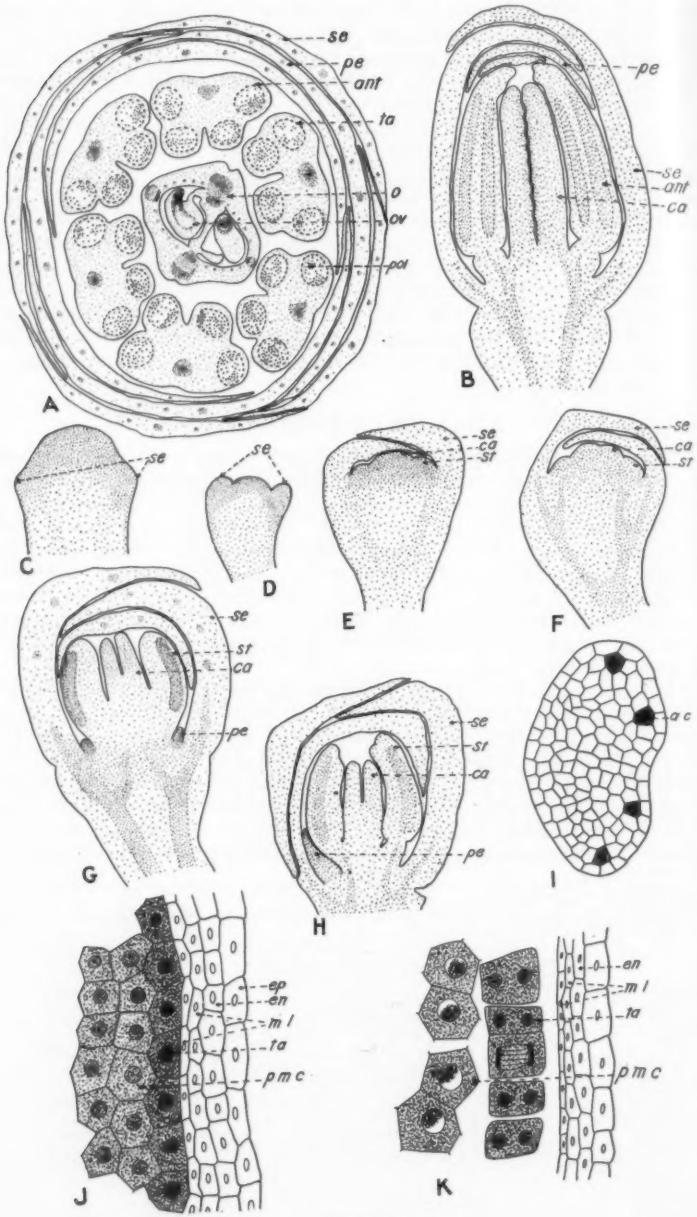

FIGURE 2.

For explanatory legend see opposite page.

The first indication of flower development in material collected from mature plants during the fall of 1930 was found in sections prepared from terminal buds killed October 28.

It is evident that the time of differentiation of flower primordia in cabbage varies with the age of the plant and the seasonal conditions. In the latitude of Washington, D.C., flower primordia in mature cabbage plants differentiate in the fall; immature plants overwintered in the open produce flower primordia in early spring.

The first indication of flower differentiation is suggested by the elongation of the entire terminal region and by the change in contour of the surface of the growing apex. Three stages in the development of the apex of the main axis are shown in figure 1. Figure 2, A and B, shows the general structure of the cabbage flower. The growing apex, at first flattened (fig. 1, A), becomes conical and soon gives rise to protuberances near the margin (fig. 2, C and D). The first whorl of swellings gives rise to the four sepals. Unless the temperature is too low for growth, the differentiation of the various organs progresses rapidly. The sepals are followed by a second whorl of swellings on the marginal surface of the apex (fig. 2, E, *st*), which produces the six stamens. The carpels are next in order of appearance and arise from the growing apex at its junction with the whorl of stamens (fig. 2, F, *ca*). The petals are the last cycle of flower organs to appear. They are first evidenced by swellings at the base of the stamens between the stamens and the sepals (fig. 2, G and H, *pe*). The succession of development of the various cycles agrees with that of *Bursa* (*Capsella*) *bursa-pastoris* (L.) Britton, in which the order deviates from the acropetalic succession characteristic of many cyclic flowers.

DEVELOPMENT OF MICROSPORANGIA

The anthers at first consist of a homogeneous mass of meristematic cells.

The anthers produce four microsporangia each. The archesporial cells which give rise to the pollen mother cells in each locule of the anther are cut off from a row of hypodermal cells under each of the four lobes. The archesporial cells are distinguishable when the anthers have developed to the stage indicated in figure 2, I. The archesporial cells divide by periclinal walls, forming an inner layer of cells that give rise to the pollen mother cells, and an outer layer that divides to produce the endothecium, one or more middle layers, and the tapetum (fig. 2, J and K). The pollen mother cells and the tapetum are readily distinguished by their difference in reaction to stains. The tapetum consists of a single layer of cells at first uninucleate (fig. 2, J, and fig. 3, B), but they soon become binucleate

EXPLANATORY LEGEND FOR FIGURE 2

A, Transverse section through cabbage flower. ×40. B, Longitudinal section of a flower in late stage of development. ×20. C, Growing apex; sepals differentiating. ×40. D, A more advanced stage of sepal development. ×33. E, Stamen differentiation. ×33. F, Differentiation of carpels. ×33. G, Carpels and petals differentiated. ×36. H, A more advanced stage of petal development. ×20. I, Transverse section through young anther showing differentiation of sporogenous cells. ×150. J, Longitudinal section through an anther in which the pollen mother cells, tapetum, middle layers, and endothecium have differentiated. ×240. K, Longitudinal section of an anther. Tapetum binucleate and pollen mother cells beginning to separate. ×240.

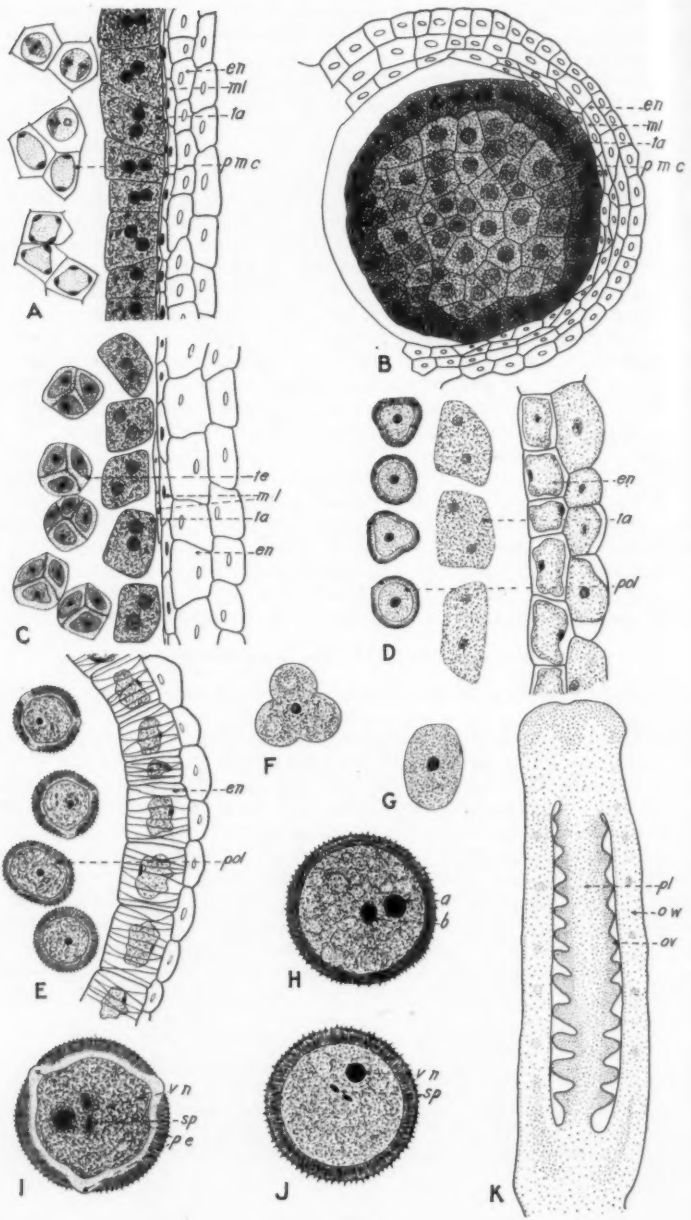

FIGURE 3.

For explanatory legend see opposite page.

(fig. 2, K). The sporogenous cells continue to divide in all planes until in transverse section the plate of pollen mother cells is 6 to 8 cells in diameter (fig. 3, B). In the homotypic division of the pollen mother cells (fig. 3, A), the spindles may extend in either opposite or parallel planes. Absorption of the middle layer or layers often begins by the time the pollen mother cells have reached the tetrad stage (fig. 3, C.) The middle layer may remain intact until the pollen mother cells are well developed, having thick, spiny exine (fig. 3, D). The variation in the absorption of the middle layer indicates the great variation in degree of many of the stages of development of the various structures. The endothecium exhibits a similar variation in time of enlargement and thickening of the cell walls in relation to the development of other layers and to pollen development. At maturity the endothecium cells are very much enlarged and have numerous radial bands of thickening in their walls (fig. 3, E).

The pollen mother cells in the tetrad stage are shown in figure 3, C. After their separation from the tetrad they are very thin walled (fig. 3, F and G); in the later stages a thick-walled spiny exine develops (fig. 3, H and J). There are three points of exit or thin areas in the exine through one of which the pollen tube emerges when the pollen grain germinates (fig. 3, I). At the time the young pollen grains are freed from the surrounding membrane in which they are held in the tetrad stage they are uninucleate. In figure 3, H, the nucleus of the pollen grain has divided to form the vegetative nucleus (*a*) and the generative nucleus (*b*). Before maturity the generative nucleus divides to give rise to the two sperms (fig. 3, I and J).

The microsporangia develop considerably before the megasporangia. Pollen cells were found beyond the tetrad stage and in some cases well developed by the time of the first division of the archesporial cells of the ovules. At the time of the first division of the megaspore mother cell the exine of the pollen grains has become thickened and the nucleus has divided to produce the vegetative and generative nuclei. The stages of development of the microsporangia and the megasporangia do not always advance at the same rate. Considerable variation in stages of development was found in material of about the same age but killed on different days.

Figure 4, E, shows a longitudinal section through an immature stigma. The stigmatic lobes are covered by long, irregular-shaped cells that produce a very rough surface. The indentations between the protruding stigmatic cells provide a lodging place for the pollen grains until they germinate and the pollen tubes have penetrated the style.

EXPLANATORY LEGEND FOR FIGURE 3

A, Longitudinal section through an anther. Pollen mother cells in homotypic division. Inner middle layer disintegrating. ×240. B, Transverse section of a single locule of an anther in same stage as figure 2, J. The various tissues have all differentiated. ×200. C, Longitudinal section through an anther. Pollen mother cells in tetrad stage. ×240. D, Longitudinal section of an anther. Pollen cells nearing maturity; middle layers have disintegrated; tapetum breaking down; cells of the endothecium beginning to enlarge. ×240. E, Longitudinal section of an anther. Pollen cells mature; tapetum entirely disintegrated and absorbed; endothecium cells much enlarged and heavily ribbed. ×240. F, Median section through a pollen grain after liberation from the tetrad, showing thin wall and three constrictions where the points of exit develop in the mature grain. ×390. G, Section through a pollen grain in the same stage as F but passing through a plane perpendicular to F. ×390. H, Median section through a pollen grain, showing vegetative nucleus (*a*) and sperm nucleus (*b*). ×390. I, Median section of a mature pollen grain through the three points of exit, showing the vegetative nucleus and the two sperms. ×390. J, Median section of a mature pollen grain in which none of the points of exit have been bisected. ×390. K, Longitudinal section through an ovary, showing the ovules developing in each of the two carpels. ×33.

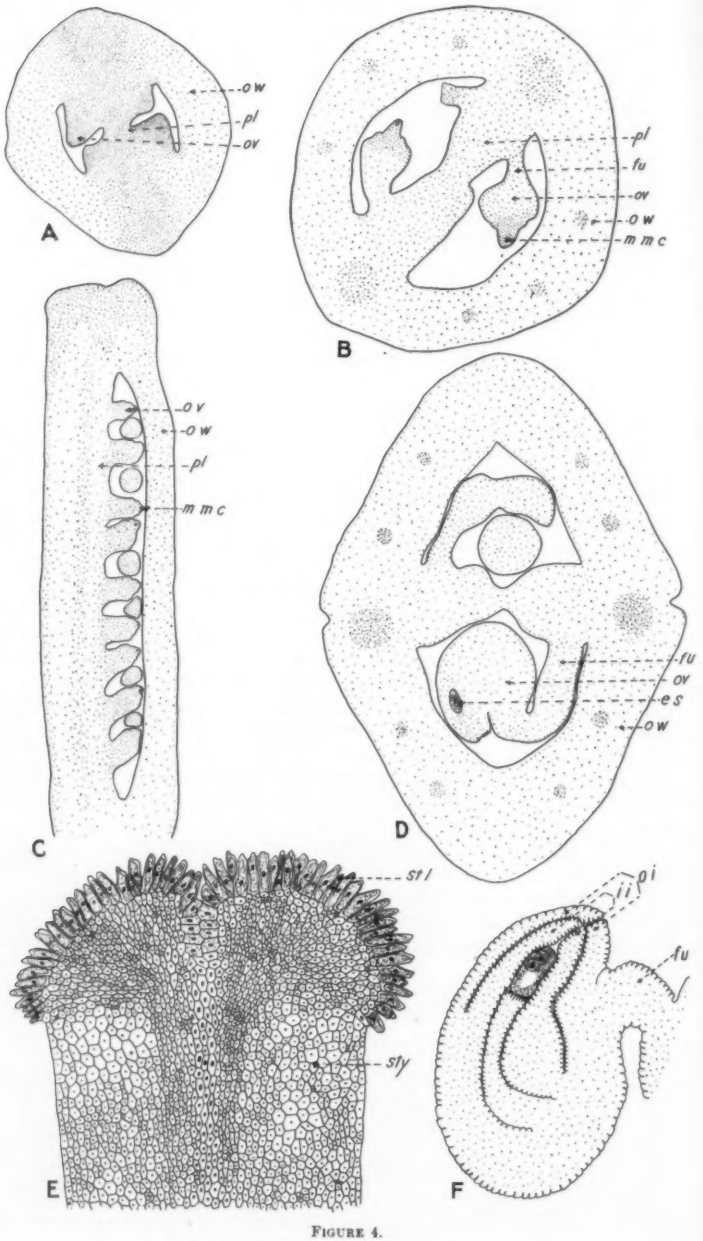

FIGURE 4.

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THE MEGASPORANGIUM AND THE EMBRYO SAC

The ovules develop from protuberances that arise from the placenta near its junction with the ovary wall. Two rows of swellings develop in each of the two carpels (fig. 3, K, and fig. 4, A). By cell division the protuberances become increasingly prominent and constitute the nucellus of the nascent ovule. When the enlarging nucellus has reached the stage indicated in figure 4, B and C, the hypodermal archesporial cell is readily distinguishable by its greater size and larger dark-staining nucleus (fig. 5, A). In some cases the archesporium is 2-celled (fig. 5, B), but only one of these cells ever becomes a megaspore mother cell. As in other Cruciferae, *Bursa* (*Capsella*) in particular, as reported by Guignard,[4] there is no parietal cell. Figure 4, D and F, shows the campylotropous structure of the ovule.

When the developing ovule has attained the stage shown in figure 5, A, the integument swellings are evident. In this case there are two, an inner and an outer integument. The two integuments are differentiated almost simultaneously, the inner one generally slightly before the outer. They develop very rapidly by cell enlargement and division and soon completely enclose the nucellus (fig. 5, C, D, and F). Figure 5, E, is a transverse section through an ovule in the same stage as figure 5, D. At this stage the nucellus and inner integument each consist of two layers of cells and the outer integument of three layers. By two successive divisions the megaspore mother cell produces a linear row of four megaspores (fig. 5, F), only one of which, usually the one toward the chalazal end, continues to function. The three micropylar megaspores soon disintegrate and are absorbed by the remaining functional megaspore (fig. 5, G), which develops rapidly and increases considerably in size before the first division of its nucleus. The nucleus is in a central position in the cell at the time of the first division (fig. 5, H). The resulting daughter nuclei separate, one migrating to the micropylar end and the other to the chalazal end of the sac (fig. 5, I). Figure 5, J, shows the four nuclei resulting from the first division of the two daughter nuclei. By two successive divisions the two nuclei divide to produce eight free nuclei (fig. 5, K). Two of the eight nuclei take a position near the center of the sac and become the polar nuclei (fig. 6, A); three migrate to the extreme chalazal end of the sac and become antipodals (fig. 6, B); the three remaining take a position near the micropylar end of the sac; the two nearest the micropyle become synergids, and the innermost one becomes the egg. The complete embryo sac at first develops most rapidly at its micropylar end (fig. 6, B). At this stage the embryo sac is pear-shaped, being much enlarged at its micropylar end and tapering toward the chalazal end. From this point on the greatest enlargement occurs at the chalazal end, while the micropylar end becomes a narrow neck (fig. 6, H and I).

[4] GUIGNARD, L. RECHERCHES SUR LE SAC EMBRYONNAIRE DES PHANÈROGAMES ANGIOSPERMES. Ann. Sci. Nat., Bot. (6) 13: [136]-199, illus.

EXPLANATORY LEGEND FOR FIGURE 4

A, Transverse section through an ovary at about the same stage of development as in figure 3, K. ×70. B, Transverse section through an ovary, showing the differentiation of the archesporial cell. ×90. C, Longitudinal section of an ovary in the same stage as in B. ×33. D, Transverse section of an ovary, showing the campylotropous ovule. ×33. E, Longitudinal section through a stigma, showing the structure of the stigmatic surface. ×90. F, Section through an ovule and its funiculus, showing the campylotropous structure and the two integuments. ×70.

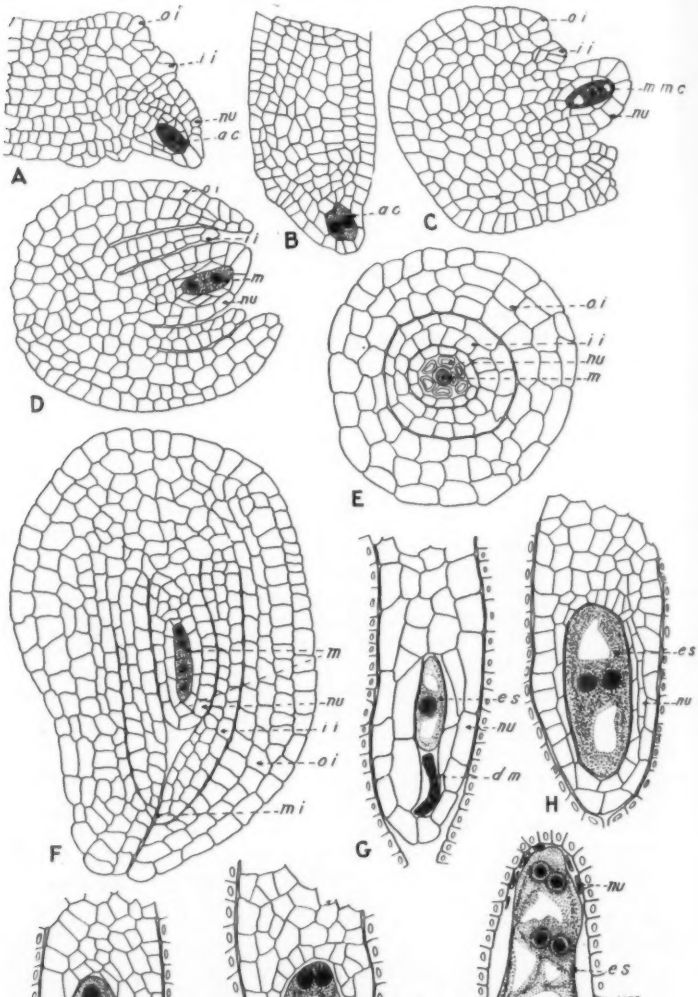

FIGURE 5.

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The sac enlarges rapidly and constitutes a very large portion of the developing ovule. As the sac enlarges, the cytoplasm becomes increasingly vacuolated. A large central vacuole soon forms, and the cytoplasm is forced to the outer wall (fig. 6, G, H, and I).

The antipodals are short-lived, soon disintegrating. They may become multinucleate before disintegration (fig. 6, D). The antipodals in this case apparently take no part in the activities of the developing embryo sac. Like the antipodals, the synergids are ephemeral and are soon absorbed by the developing sac. Many cases were noted in which the embryo sac and the nucellus had not developed and fertilization had evidently failed to take place. However, the integuments had continued to enlarge by cell division, and the ovules in some cases were as large as the fertilized ovules, but in the later stages these unfertilized ovules shriveled and died. The reason for the failure of certain egg cells to be fertilized was not determined.

As shown by figure 6, C and D, the pollen tube may enter from either the micropylar or the chalazal end of the sac. In most cases the tube was found to enter from the micropylar end.

The structure of the cabbage flower is such that under field conditions the ovules may be either selfed or cross-fertilized. The flowers are visited by various insect species. When the seed-producing plants are grown in the open and unprotected from insects, the percentage of cross-fertilization is likely to be quite high. Where self-pollinated seed is desired it is necessary that the plants be protected from insects. The statement is often made that a single cabbage plant rarely produces seed, or is self-sterile. Some strains are doubtless largely self-sterile, but this is not true of all. In this study no difficulty was experienced in obtaining fertile ovules that matured normal seed when the flowers were artificially selfed.

EMBRYO DEVELOPMENT

There appears to be some variation in the time elapsing between pollination and fertilization. Considerable variation was noted in stages of development of ovules. Within a single carpel ovules were found in which the endosperm had completely lined the wall of the embryo sac; in other ovules in the same carpel fertilization had not yet taken place. The first fertilized eggs were found in material killed 4 days after pollen had been applied to the stigmas, but in most cases fertilized eggs and pollen tubes within the sac were found in material killed 5 or 6 days after pollination. A wall is formed around the egg soon after fertilization. The synergids disintegrate about the time the egg is fertilized (fig. 6, E). The endosperm nucleus divides slightly in advance of the fertilized egg (fig. 6, E). The fertilized egg cell, by several successive divisions, produces a linear row of cells (fig. 6, F and H). The filament usually consists of 6 or 8 cells before the terminal or embryo cell divides (fig. 6, H, I, and J).

EXPLANATORY LEGEND FOR FIGURE 5

A, Section through a young ovule, showing the archesporial cell and the developing integuments. ×180. B, Section through an ovule, showing 2-celled archesporium. ×150. C, Section through a young ovule just before the first division of the sporogenous cell. ×120. D, Ovule showing development of integuments after the first division of the sporogenous cell. ×120. E, Transverse section through an ovule in the same stage as D. ×120. F, Ovule showing the four megaspore mother cells resulting from division of the archesporial cell shown in A. ×120. G, Section through an ovule, showing disintegration of the three nonfunctioning megaspores. ×180. H, Embryo sac after the first division of its nucleus. ×180. I, Embryo sac; the two nuclei have moved to opposite poles. ×180. J, Embryo sac in 4-nucleate stage. ×180. K, Embryo sac with eight nuclei. ×180.

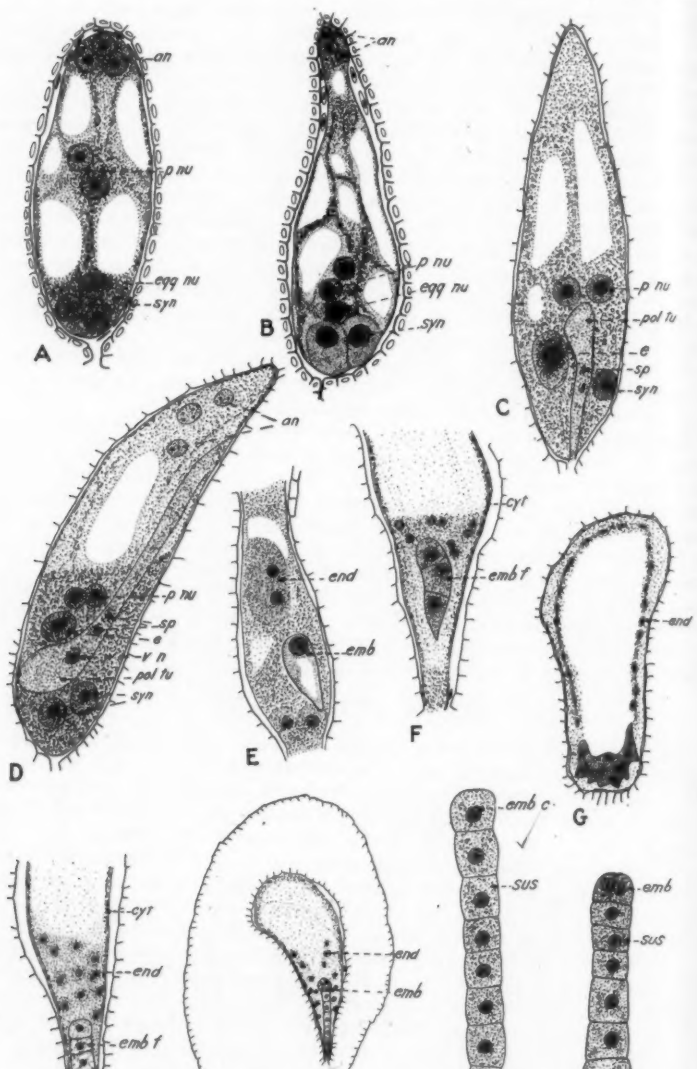

FIGURE 6.

For explanatory legend see opposite page.

In this discussion the terminal cell of the filament is considered as the "embryo cell," although, as in *Bursa*, the first cell of the suspensor contributes to the embryo tissues. Some of the cells of the suspensor filament undoubtedly continue to divide by transverse walls after the first division of the embryo cell, for never more than 8 cells were found in the filament at this stage; yet in later stages in the embryo development suspensors were found with 10 and 12 cells. The first division of the embryo cell is longitudinal (fig. 6, K). The two daughter cells by longitudinal and transverse walls divide to produce the 8-celled or octant stage (fig. 7, A). The first transverse walls separate the cotyledonary from the hypocotyledonary regions of the embryo. The cotyledons and the epicotyl develop from the four apical octants shown in figure 7, A, *a*. As in *Bursa*, a portion of the hypocotyl is derived from the hypophysis, which is cut off from the suspensor by a transverse division of the cell adjacent to the embryo (fig. 7, C). The hypocotyl then is derived from the portion *b* and a part of cell *c* in figure 7, A.

The dermatogen is differentiated early in the development of the embryo (fig. 7, B), except for that portion derived from the hypophysis, and is soon completed. The plerome and periblem differentiate when the embryo has reached the stage indicated in figure 7, C. The portion of the embryo *a* in figure 7, A, develops more rapidly than *b*, as indicated in figure 7, D and E. The suspensor cell adjacent to the embryo has divided by a transverse wall. The daughter cell next to the embryo contributes to the periblem and dermatogen of the rootcap; the other plays no part in the embryo development but remains as part of the suspensor.

The first division of the hypophysis is transverse. The daughter cell adjacent to the embryo contributes to the periblem of the root (*a*) in figure 7, D; the basal daughter cell (*b*) produces a plate of four cells by two longitudinal divisions. The longitudinal wall in cell *b* precedes the longitudinal division of cell *a* in figure 7, E. A transverse division of the plate of four cells in *b*, figure 7, F, results in a second plate of four cells *c* in figure 7, G. The four cells in *b*, figure 7, G, complete the dermatogen of the root tip. The dermatogen, which is the first tissue to be differentiated, is the last to be completed. The cells in (*c*) of figure 7, G, become the first layer of the rootcap shown in figure 7, H. By transverse divisions of this layer of cells other layers are added to complete the rootcap, as indicated in figure 7, I and J. The development of the embryo follows very closely that reported by Hanstein [5] and Famintzin [6] for *Capsella*.

[5] HANSTEIN, J. ENTWICKLUNGE DES KEIMES DER MONOKOTYLEN UND DIKOTYLEN. *His* Botanische Abhandlungen aus dem Gebiet der Morphologie und Physiologie, Bd. 1, Heft 1, 112 p., illus. Bonn. 1870.

[6] FAMINTZIN, A. EMBRYOLOGISCHE STUDIEN. Mem. Acad. Imp. Sci. St. Petersbourg, 26, no. 10. 1879.

EXPLANATORY LEGEND FOR FIGURE 6

A, Embryo sac; the polar nuclei have moved to the chalazal end of the sac and the synergids toward the micropyle. ×180. B, Embryo sac just before the disintegration of the polar nuclei. ×180. C, Embryo sac in which the pollen tube has entered from the micropylar end. ×180. D, Embryo sac in which the pollen tube has entered from the chalazal end. Antipodals multinucleate. ×180. E, Embryo sac after fertilization. The endosperm nucleus has completed its first division; synergids disintegrating. ×200. F, Embryo sac with embryo in 3-celled stage. From material killed 5 days after pollination. ×200. G, Embryo sac showing mass of endosperm at chalazal end. ×40. H, Embryo in the 6-celled filament stage. From material killed 8 days after fertilization. ×120. I, Section through an ovule, showing embryo in the octant stage. Killed 10 days after pollination. ×33. J, Embryo filament before division of the terminal or embryo cell. Killed 8 days after pollination. ×200. K, First division of embryo cell. Killed 8 days after pollination. ×200.

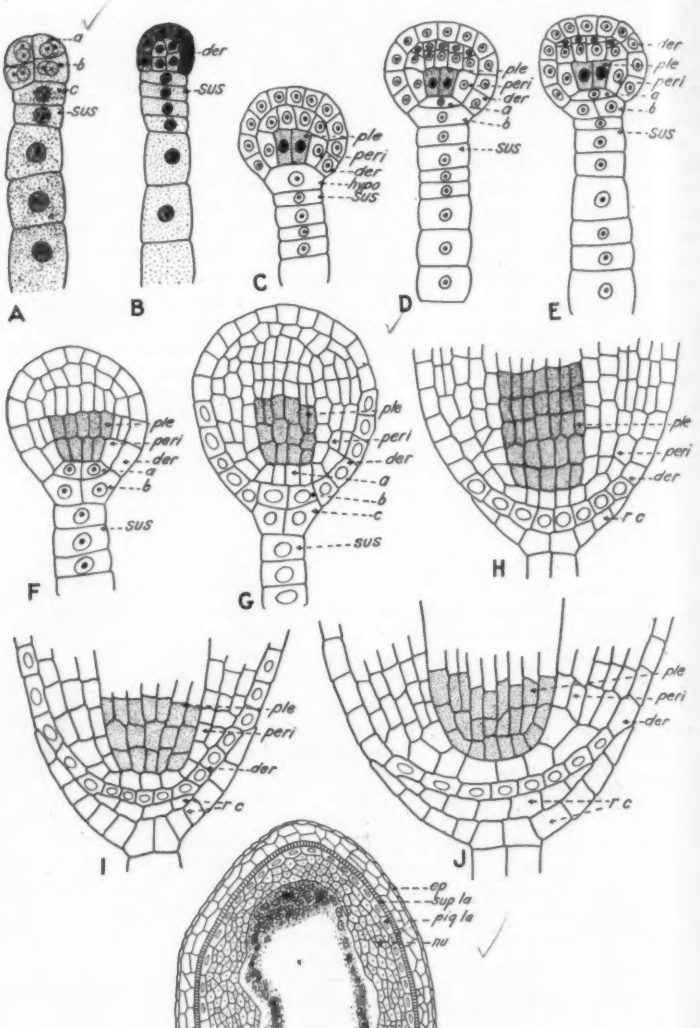

FIGURE 7.

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THE ENDOSPERM

The primary endosperm nucleus divides before the egg. Further divisions of the endosperm follow in rapid succession, hence it develops in advance of the embryo.

Many of the nuclei divide simultaneously, as in some sections nearly all of the nuclei present were in some phase of cell division. The nuclei are at first free without separating walls. As the embryo develops, the endosperm surrounding the embryo becomes separated by walls, while some of the nuclei at the chalazal end apparently never become separated. As the vacuolation of the sac increases and the cytoplasm is driven outward against the wall of the sac, the endosperm forms a single cell layer around the wall of the sac and is embedded in the limited cytoplasm. Generally, a considerable mass of endosperm develops at the extreme chalazal end of the sac (fig. 6, G), and to a less extent around the developing embryo (fig. 7, K, and fig. 8, A). No very large amount of endosperm tissue ever develops, and that which does develop is soon absorbed by the growing embryo; hence the seed of cabbage is without endosperm.

THE SEED

The embryo constitutes the largest portion of the mature ovule or seed (fig. 8, B and C). As the embryo develops and expands it fills the large vacuole of the embryo sac and absorbs the cytoplasmic contents of the sac as well as the endosperm. At maturity the endosperm has disappeared and most of the nucellar tissue has been absorbed. The stored nutrient materials for the young seedling at the time of germination are carried by the cotyledons and hypocotyl of the embryo. Surrounding the well-developed embryo are the seed coats derived from the two integuments and a layer or two of cells next to the embryo, which are all that remain of the nucellar tissue. The outer coat of the seed consists of the epidermis and in most cases two layers of subepidermal tissue which is derived from the outer integument; the inner coat consists of two layers, a heavy-walled supporting tissue one cell in thickness and an irregular layer of pigmented cells originating in the inner integument. In addition to these layers and surrounding the embryo are one or more layers of cells remaining from the once many-celled nucellus, most of the nucellar tissue having been absorbed by the embryo.

EXPLANATORY LEGEND FOR FIGURE 7

A, Embryo in the octant stage. Killed 10 days after pollination. ×200. B, Embryo showing differentiation of dermatogen. Material killed 10 days after pollination. ×200. C, Embryo showing differentiation of plerome and periblem. The first suspensor cell has divided; the daughter cell next to the embryo contributes to the embryonic tissues. Killed 11 days after pollination. ×200. D, Embryo in which the periblem of the root has been completed from the hypophysis. Killed 11 days after pollination. ×200. E, Embryo in which basal daughter cell from first division of the hypophysis has divided longitudinally. ×200. F, Embryo in which hypophysis daughter cell adjacent to embryo has divided by longitudinal walls. ×200. G, Embryo in which the dermatogen of the root tip has been completed. Killed 15 days after pollination. ×200. H, Embryo in which one layer of the rootcap has developed. Killed 17 days after pollination. ×200. I and J, Later stages of embryo development (19 and 21 days after pollination) in which two layers of rootcap have developed. ×200. K, Section through an ovule, showing development of the cotyledons. Pigmented layer differentiating. ×40.

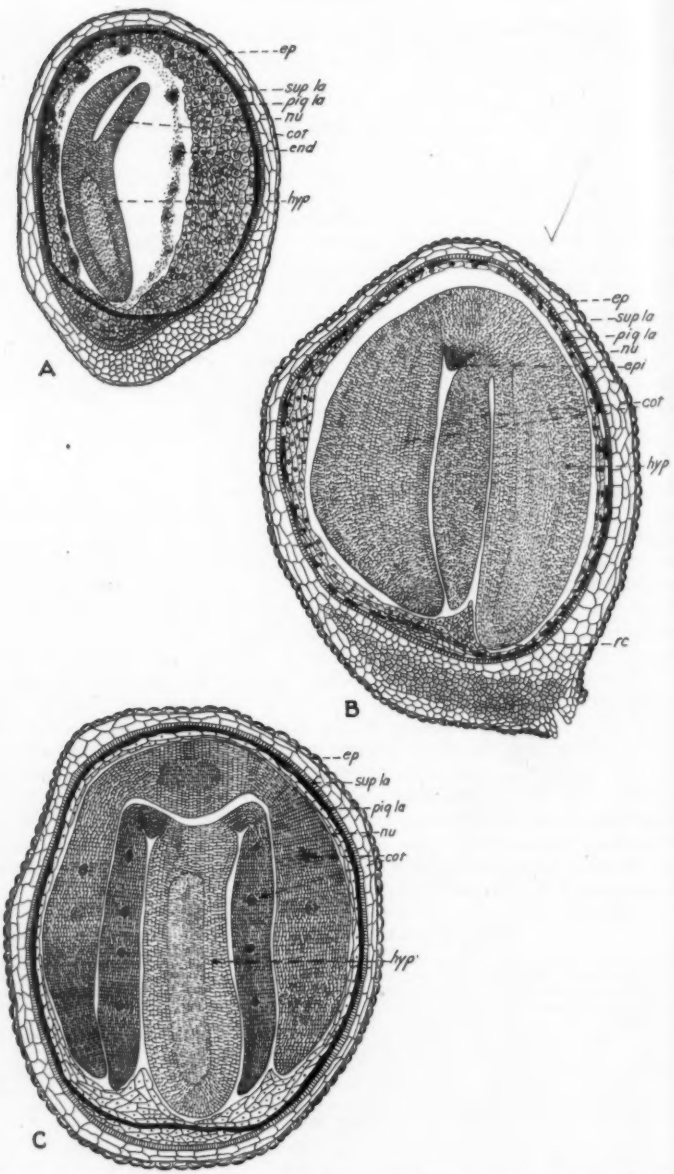

FIG. 8—A, Section through an ovule, showing development of the hypocotyl. ×40. B, Section through an ovule just before maturity. Cotyledons and hypocotyl well developed; the epicotyl differentiated. ×40. C, Section through a seed near maturity. ×40.

SUMMARY

The differentiation of flower primordia in cabbage varies with the age of the plant and conditions of the environment in which it is placed. Young seedlings overwintered in the open at Washington, D.C., were found to differentiate primordia during the latter part of February or early March. Mature plants that have formed a head differentiate flower primordia soon after cold weather sets in, or about the latter part of October.

The order of appearance of the various cycles of floral organs deviates from the usual acropetalic succession. The four whorls of organs appear in the order of sepals, stamens, carpels, and petals. In this respect they agree with the order reported for *Bursa* (*Capsella*) *bursa-pastoris* (L.) Brit.

The microsporangia are derived from a single row of hypodermal cells under the epidermis of each lobe of the 4-lobed anther. The primary sporogenous cell divides by a periclinal wall cutting off a primary parietal cell toward the epidermis. The primary parietal layer by periclinal walls divides to form the endothecium, one or more middle layers, and the tapetum. The innermost daughter cell from the first division of the primary sporogenous cell divides to form a plate of pollen mother cells 6 to 8 cells in transverse section. The microsporangia develops before the megasporangia.

The megasporangium is derived from a hypodermal cell of the nucellus. In some cases the archesporium is 2-celled, but only one of these cells functions as a megaspore mother cell. No parietal layer develops in the megasporangium. Two rows of ovules develop in each of the two carpels. The two integuments differentiate almost simultaneously; they appear early in the development of the ovule, develop rapidly, and soon completely enclose the nucellus. The one functioning cell of the archesporium divides to produce a linear row of 4 megaspores, 3 of which fail to develop, disintegrate, and are absorbed by the functioning megaspore, usually the one toward the chalazal end.

The antipodals and synergids are short-lived and are absorbed soon after fertilization.

The time elapsing between pollination and fertilization varies, but fertilization in most cases probably occurs about 5 days after the pollen is applied to the stigma. Numerous ovules were found in which fertilization had apparently not taken place, as indicated by the failure of the embryo sac and the nucellus to develop and the shriveling of the ovules in late stages.

The primary endosperm nucleus divides slightly in advance of the egg. The later divisions follow in rapid succession, as indicated by the many endosperm cells in an ovule found dividing simultaneously. Considerable endosperm develops at the chalazal end of the sac and around the embryo in its early stages. Only a single-celled layer of endosperm lines the major portion of the embryo sac, and this is absorbed by the developing embryo before its maturity.

The larger portion of the mature ovule consists of the embryo. The mature embryo is surrounded by several layers of tissue derived largely from the two integuments. The outer seed coat consists of the epidermis and two layers of subepidermal tissue derived from the outer integument. The inner seed coat consists of a heavy-walled

layer of supporting tissue and a pigmented layer a single cell in thickness just beneath the heavy-walled supporting layer, both of which originate in the inner integument. One or more layers of nucellar tissue may surround the mature embryo. Most of the nucellus is absorbed by the embryo before maturity.

The development of the embryo follows very closely that reported for *Bursa* (*Capsella*). The first transverse division of the embryo cell or proembryo separates the cotyledonary from the hypocotyledonary portions of the embryo. A portion of the embryo is derived from the hypophysis, which is cut off by a transverse wall from the first suspensor cell next to the embryo cell.

THE INHERITANCE OF SOME PLANT COLORS IN CABBAGE[1]

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INTRODUCTION

Until recently, only two genetic colors of *Brassica* were recognized, red or purple, and green. In 1924 Kristofferson called attention to a type with colored midribs which probably is the same as the type herein described as sun color. The appearance of a previously undescribed color type called magenta, in the cultures of the junior author immediately aroused interest in its genetic relationship to the previously described color types. The purpose of this paper is to report the results of investigations on the genetic relationships between color types which have been designated as purple, magenta, sun color, and green.

REVIEW OF LITERATURE

As early as 1928 Wiegmann (*9*)[3] reported that red color in cabbage was incompletely dominant over green. E. P. F. Sutton (*8*), Pease (*5*), and Allgayer (*1*) also reported incomplete dominance in the F_1 and concluded that only a single factor was involved in the production of the red color.

In a later paper Pease (*6*), from his study of a blue kohlrabi × green savoy cabbage cross, concluded that the purple of blue Vienna kohlrabi was due to two complementary factors. Moldenhawer (*4*) obtained similar results from a purple kohlrabi × green brussels sprouts cross.

A. W. Sutton (*7*) reports plants with purple foliage in the F_2 of Drumhead cabbage × kohlrabi and Drumhead cabbage × thousand-headed kale crosses, although no mention was made of color on the F_1 or on either of the parents.

Kristofferson (*3, pt. I*) reports on the inheritance of four color types in crosses between cabbage, brussels sprouts, kale, and broccoli. The type found in red cabbage was described as "dark red violet" throughout all parts of the plant. There were also three types with green blades but differing in the color of the middle vein. "This is of the same dark red violet color as the rest of the leaf in red cabbage,

[1] Received for publication Feb. 18, 1933; issued August 1933.

[2] The senior author wishes to acknowledge his indebtedness to Dr. H. C. Thompson, of the Department of Vegetable Crops, Cornell University, for providing space and labor involved in growing part of the cultures of 1929; to Dr. John Bushnell, of the Ohio Agricultural Experiment Station, for making the final check on the 1929 progeny; and to the administrative officials of the Ohio Agricultural Experiment Station who provided the facilities necessary for the conduct of this study and permitted the writer to pursue this investigation in connection with his other duties. The inbred stocks of the color types and seed for the F_1 and F_2 populations of the original crosses were supplied by the junior author.

[3] Reference is made by number (italic) to Literature Cited, p. 247.

light red (violet) in cabbage and brussels sprouts, and green in kale and broccoli."

On the basis of F_1 and F_2 results, he tentatively assumed four factors, A, B, C, and D, to be involved, with the following effects: A produces no color by itself but when present with B the combination causes a dark-red violet coloration of the midrib; B causes a light-red coloration of the midrib; C causes no coloration itself, but with A it produces dark-violet midribs in kale; D causes the general dark-red color of red cabbage.

He experienced considerable difficulty in his classification of midrib colors and in a later report (*3, pt. II*) admits that "for a firm establishing of the factorial basis of the total dark red violet color it may be necessary to grow the F_3 generation."

PLAN OF PROCEDURE

In order to secure one generation per year, seed was sown about the middle of May, the plants were set 18 by 36 inches apart in the field by July 1 and the mature heads harvested about October 15. These were stored at 40° F. until the middle of December, when they were placed in 10-inch pots and grown in the greenhouse at a temperature of 60° to 70°. Flowering occurred during February and March, and seed was mature by May 1.

Glassine bags large enough to inclose the inflorescence were used to prevent any chance cross-pollination that might occur in moving the plants to and from the bench for selfing or crossing. Only the terminal raceme was allowed to develop, as it normally provided sufficient seed if properly pollinated and was much easier to handle without breaking than a plant with several racemes.

Self-pollination was effected by removing an anther or anthers with a pair of curved steel forceps and then touching the anther to the stigmas of open flowers on the same plant. Before working on another plant, the forceps and tips of the fingers were dipped in 95 percent alcohol to kill adhering pollen grains. Flowers to be crossed were emasculated 1 day before they would normally open and were pollinated the following day.

Due to shortage of space in the field, several thousand seedlings were grown in flats in the greenhouse during the summer of 1929. Crowding of plants and high temperature accompanied by periodic water shortage resulted in extreme hardening and a high production of pigment. Readings on these plants were made in September, at which time most of the plants had formed a small rosette and enough of a terminal bud so that purple could be readily distinguished from sun color. Where other data were available to serve as a check, these figures are included in the results even though the proportions of different color types are not exactly like those of the field-grown material.

No attempt was made to classify the field-grown plants for color until after heads had formed, which was usually during September. The color type of each plant was checked on at least two different dates. Plants of the parental lines were grown each year for comparison purposes. The F_2 and some of the other families were grown during two different years.

SOURCE AND DESCRIPTION OF COLOR TYPES

PURPLE TYPE

The purple type is of ancient origin and is represented by several horticultural so-called red varieties. As far as the authors have been able to ascertain from a study of the descriptions given, all the genetic data to date have been concerned with this color, although the word "red" has commonly been used to describe it. All of the American varieties of so-called red cabbage are of this type.

Practically all plants of this color have a heavy "bloom", i.e., are glaucous, which somewhat obscures the undercolor and gives the leaves a dull, gray-purple appearance. Moreover, the presence of chlorophyll in the cells containing the pigments adds to the difficulty of accurately determining the color by the use of any of the available color standards. According to Ridgway,[4] when examined in diffused light the homozygous purple type with undisturbed bloom may be described as dull dusky purple or naphthalene violet. When the waxy bloom is removed the color most nearly agrees with raisin purple or blackish purple.

The intensity or depth of the purple color increases with the maturity of the plant and is also increased by unfavorable growth conditions such as drought or cold weather. It is present as soon as the seedlings appear above ground although somewhat obscured by the green of the chlorophyll. Purple can be distinguished from magenta with a fair degree of accuracy in the early seedling stages and with almost complete certainty if the young plants are hardened by exposure to low temperatures or drought conditions.

Light is not essential for its formation. It is found throughout the mature head along the midribs and larger veins and more or less diffused between the veins.

When heterozygous for both factors assumed to be responsible for the production of purple, the plant is not so dark a purple as is the homozygous plant, and when the outside leaves of the head are stripped away it can be observed that the purple color is very largely limited to the midribs and larger veins of the leaf. The spaces between veins are white or light green.

MAGENTA TYPE

The term "magenta" was applied by the junior author to a distinct red type in the progeny of a selfed plant of the Danish Round Red variety. Approximately 25 percent of the progeny were magenta, the balance being purple like the parent plant.

With the bloom present the color on the magenta stems and outer leaves matches fairly well with Ridgway's daphne pink or daphne red. In seedlings and heterozygous plants the color is more dilute and varies between light Persian lilac and Persian lilac in tint. The color on the interior of the head where chlorophyll and bloom are practically absent agrees fairly well with Ridgway's spinel red. This type could more properly be called red than the above-described purple type, but to avoid confusion, the term "magenta" has been selected.

[4] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D.C. 1912.

Like the purple type, the color of this type develops on the stem and leaves inside the cabbage head and is similarly affected by drought and other unfavorable growth conditions. The color is of clearest hue on the bud leaves of young plants or on the leaf base or stem at the base of the mature head. Magenta seedlings may be distinguished when very young from purple ones by holding the young plants between the observer and the source of light or against a sheet of white paper.

In the field, plants that are heterozygous for the magenta factor may on superficial examination be confused with those of the green type because the pink color does not always extend to the tip of the leaf and is obscured by the bloom and chlorophyll color.

In homozygous plants the magenta color is diffused between the veins on the leaves inside the head, whereas in heterozygous plants the color is very largely limited to the midribs and larger veins.

SUN-COLOR TYPE

The term "sun color" was selected for the sun-color type because it develops color only on the portions of the plant exposed to the light. This character is commonly found on horticultural varieties of late cabbage and was isolated in homozygous condition by selfing a commercial variety. The color is of the purple type described above and in homozygous plants is found on the exposed midribs, larger veins, along the serrulate or dentate edge of all leaves and diffused between the veins on the outside leaves of the head. It also occurs on the stem when exposed to sunlight. There is very little formation of color in rapidly growing plants of this type and in a normal season it does not develop to the fullest extent until the cool, frosty nights of autumn. Unfavorable growth conditions apparently favor its development even in small seedlings. If the heads mature without encountering cool nights or unfavorable growth conditions, the color may not be sufficiently well developed to permit accurate classification of the plant. In this event, the small heads formed from axillary buds later in the season have been found to provide an accurate index of the plant's color type.

Because of the effect of environmental factors on its expression the sun-color type varies more widely than purple or magenta. In plants heterozygous for this character the color may be limited to a faint purpling of the midrib and the serrulate or dentate margin of the leaves. In other plants from the same family it may be necessary to strip back the outside leaves of the head to distinguish it from heterozygous purple. The leaves and stem of the interior of the head of a sun-color plant are white or very light green, and stripping the head leaves or examining the stem at the base of the head was used as a method of distinguishing it from purple or magenta.

GREEN TYPE

The green type is light green and, except under unusually unfavorable growing conditions, free from any other color on any part of the vegetative plant.

HISTOLOGY OF COLOR DEVELOPMENT

In all the above-described color types, microscopic examination of free-hand sections showed the color (other than green) to be limited to 1 and not more than 2 layers of the nearly round cells of the spongy mesophyll adjacent to the layer of colorless cutinized epidermal cells. The red and purple pigments are contained in the cell sap and are not due to chromoplastids.

EXPERIMENTAL DATA

The probability values in tables 1 and 3 were obtained by direct interpolation from the values given by Fisher (*2, table III*). When the P value is less than 0.05 it is unlikely that the deviations are due to chance alone, and one must look with suspicion on the hypothesis advanced, or account for the deviation in some other way than errors of random sampling.

Although two crosses of an entirely different nature were carried simultaneously, the one involving all the color types will be considered first since it provided all the data necessary for an analysis of the above-described colors.

A cross was made between sun-color and magenta plants and will hereafter be referred to as cross no. 328. The F_1 plants of this cross were all purple, a new type, which at once suggested the interaction of two factors. The F_2 results given in table 1, group 1, show that the parental types were recovered as well as the purple of the F_1 and a pure green type. The distribution of plants in the 4 classes gives a very good fit to the dihybrid ratio of 9:3:3:1, $P=0.86$. Deviations as large as these might therefore be expected through chance alone in about 6 out of 7 trials.

TABLE 1.—*Progenies of succeeding generations from selfed plants of purple, magenta, and sun color found in crosses 328 (magenta × sun color) and 327 (magenta × green)*

Group no.	Number of families	Number of plants				Total number of plants	Calculated ratio	P
		Purple	Magenta	Sun color	Green			
F_2 PROGENIES OF F_1 PURPLE FROM CROSS 328								
1	2	406	144	134	42	726	9:3:3:1	0.86
F_3 PROGENIES OF F_2 PURPLES FROM CROSS 328								
2	28	1,153	384	352	109	1,998	9:3:3:1	0.22
3	6	261		78		339	3:1	.41
4	3	114	40			154	3:1	.78
5	6	190				190	All	
F_4 PROGENIES OF F_3 PURPLES FROM CROSS 328								
6	7	85	28	27	13	153	9:3:3:1	0.71
7	1	12	4			16	3:1	
8	1	14		6		20	3:1	.62
9	2	69		32		101	3:1	.13
10	4	148				148	All	
11	3	64	24			88	3:1	.64

TABLE 1.—*Progenies of succeeding generations from selfed plants of purple, magenta, and sun color found in crosses 328 (magenta, × sun color) and 327 (magenta × green)*—Continued

F_3 PROGENIES OF F_1 MAGENTAS FROM CROSS 327

Group no.	Number of families	Number of plants				Total number of plants	Calculated ratio	P
		Purple	Magenta	Sun color	Green			
12	3		599		192	791	3:1	0.65

F_3 PROGENIES OF F_2 MAGENTAS FROM CROSSES 328 AND 327

13	9		254		75	329	3:1	0.37
14	4		[a] 116			116	All	
15	3		50		9	59	3:1	.09
16	6		115			115	All	

F_4 PROGENIES OF F_3 MAGENTAS FROM CROSSES 328 AND 327

17	5		109		44	153	3:1	0.29
18	2		28			28	All	
19	1		4		3	7	3:1	.28
20	1		14			14	All	
21	1		22			22	All	
22	2		34		11	45	3:1	.94
23	2		42			42	All	
24	2		49			49	All	

F_3 PROGENIES OF F_2 SUN COLORS FROM CROSS 328

25	10			[b] 272	95	367	3:1	0.70
26	4			224		224	All	

F_4 PROGENIES OF F_3 SUN COLORS FROM CROSS 328

27	2			68		68	All	
28	2			10	4	14	3:1	0.76

[a] Plus 8 purple plants.
[b] Plus 1 magenta plant in 1 family, 9 purples in another family, and 6 purples in still another family.

One of the F_1 purple plants was back crossed to a pure green plant, and the progeny were distributed in approximately equal numbers in the four color classes as shown in table 3, group 1. The fit of expected to observed on the basis of a 1:1:1:1 distribution is very good, *P* equaling 0.83.

These data seemed sufficient to postulate that two independently inherited factors were involved in the production of the purple color.[5] The symbol M has been assigned to the factor responsible for the production of magenta and S to that for the production of sun color.

Because of the labor involved in securing hybrid seed in large numbers, the number of F_1 plants in many test crosses is rather small. It was much easier to secure large numbers of seed by selfing, and

[5] Kwan, (Chia Chi), in a personally published abstract which is filed with the Graduate School of Cornell University, as of June 1933, reports the results of crosses between purple and sun red and purple and green cabbage. The material for the crosses was furnished by the junior author. Kwan found the purple to be incompletely dominant in the F_1 to sun red and concludes that purple is produced by duplicate genes which are cumulative in effect. Using the same purple in a cross with green, he found the F_1 to be intermediate in color and the F_2 to be composed of approximately 9 purple: 3 sun red: 4 green. F_3 results substantiate the hypothesis that the purple and green parents differ by two factor pairs, designated as Gg and Hh. G is a basic factor for pigment which with H gives purple and with h gives sun red. Plants with g are green. It is evident that the purples used differ genetically from those employed in the present study.

more attention has therefore been paid to growing successive filial generations as a test of the genetic nature of the plant than to back crossing it to the double recessive green type or to known testers.

BEHAVIOR OF F_2 COLOR TYPES IN LATER GENERATIONS

Purple Type

Thirty-six of the purple plants from the F_2 of cross 328 were grown and selfed in the greenhouse. Assuming that purple is due to the action of two independent factors, one would expect to find four genetic types of purples in the F_2.

The F_3 progeny results given in table 1, groups 2 to 5, inclusive, show that all four expected genotypes were present and the distribution of color types in each group agrees well with the theoretical expectation as indicated by the probability value. Goodness of fit of the proportions of expected genotypes was not calculated as the sample was not a random one, i.e., plants with different degrees of purple pigmentation in the F_2 were selected in proportion to the expected number of each based on a guess as to their genotype.

Selections were made of all the color types present in each of the F_3 groups represented in table 1. The F_4 results from the F_3 purples are given in groups 6–11 inclusive. Families in groups 6, 7, 8 represent selections from group 2, those in groups 9 and 10 from group 3, and those in group 11 from group 4. None of the plants from the pure-breeding group 5 survived to produce progeny. In view of the small number of plants involved it is not surprising that an F_3 plant producing only purple plants should fail to be present in those selected from groups 2 and 4. The distribution of color types in the segregating families was reasonably close to the expected proportions and corroborates the postulated genotype for the F_2 plants.

Magenta Type

For convenience in presenting the results, the data from the second cross (327) mentioned at the beginning of the section on experimental data, will be included in the results with the magentas from the 328 cross. Cross 327 was made between magenta from the same family as that used in the 328 cross and a green from a family that had been breeding true for this type for several generations.

According to the genotypes postulated, only two kinds of magentas would be present in the F_2, those (ssMM) which in the F_3 would produce only magenta and those (ssMm) which would produce magenta and green in an approximate 3:1 ratio. Such was the case, as the data in table 1 show. The eight purple plants found in one family may be explained by assuming that a tag was lost from a crossed pod and this pod later harvested with the selfed ones.

The following array shows the relation between the F_4 families and the F_3 families:

F_3 group 13 represented by F_4 progeny in group (s) 17.
F_3 group 14 represented by F_4 progeny in group (s) 18.
F_3 group 2 represented by F_4 progeny in group (s) 19, 20.
F_3 group 4 represented by F_4 progeny in group (s) 21.
F_3 group 15 represented by F_4 progeny in group (s) 22, 23.
F_3 group 16 represented by F_4 progeny in group (s) 24.

In only one case (group 17) did the expected types fail to appear, but in this sample of only 5 plants it is not surprising that both genotypes were not present.

Sun-Color Type

Fourteen of the F_2 sun colors were selfed and produced an F_3 generation. On the basis of the proposed hypothesis one would expect 2 Ssmm to 1 SSmm. The actual ratio as shown in table 1, groups 25 and 26, is 10:4, a close approximation of that expected. The presence of purple or magenta plants in three of the families was probably due to accidental crosses or to the loss of tags from crossed pods.

The F_4 results given in group 27 of table 1 show that these families, selected from a family in group 26, produced only sun-color plants as expected. The F_3 sun-color plants whose progeny results are shown in group 28 must have been genotypically Ssmm. They were selected from group 2 of table 1.

Green Type

No difficulty was experienced in separating sun color from green in the F_2 progeny of cross 328 in the fall of 1927 at Wooster. In a culture of the same F_2 grown in the gardens of the Plant Breeding Department, Cornell University, during the same year, the proportion of green plants was much lower than in the Wooster culture and raised the question as to the correctness of the Wooster classification. Nine green plants were accordingly selfed and the F_3 progeny grown in 1928 and 1929.

In 1928 the growing conditions at Wooster were very similar to those of 1927 at Ithaca in that the latter part of the summer was marked by a severe drought accompanied by high temperatures. These conditions were apparently responsible for the development of a small amount of pink color on the midribs of a large number of plants in progeny which were expected to contain only green plants. This color compared in shade more closely with magenta than with sun color and was limited almost exclusively to the middle section of the midrib of the leaves immediately surrounding the head. This type was recorded as questionable sun color. The most intense coloration on sun-color plants, on the other hand, frequently occurred on the upper third of the midrib and along the edge of the leaf. The difference in location and shade of color made it possible to easily separate most of the questionable type from the sun color after these differences were realized. Failure to differentiate accurately between these types in the beginning may account in part for some of the wide deviations from the expected ratios.

The 1929 season was also characterized by hot dry weather during August and the first half of September, which resulted in the formation of pink midribs on many otherwise green plants. Timely and abundant rainfall during the later half of the growing season resulted in rapid growth and the disappearance of this color from some plants. Pease (*5*) mentions the fact that hot dry weather in England resulted in the formation of light-red or pink color on the leaves of plants that under normal conditions were pure green.

The F_3 results from F_2 greens for each year are given in table 2, group 1, and it will be noted that whereas in 1928 many of the plants

were thought to be sufficiently pigmented to justify a classification of sun color, in 1929 only one plant was so recorded. Part of this shift may have been due to a clearer conception of the differences between sun color and this questionable type as a result of the 1928 experience.

The F_4 progenies (group 7, table 2) of six of these F_3 plants recorded as sun color show that they were incorrectly classified, since none of them produced sun color in the F_4. Data to be given on intercrosses between F_2 color types support this conclusion.

TABLE 2.—*Progenies of succeeding generations from selfed plants of green, questionable sun color, and sun color from crosses 328 (magneta × sun color) and 327 (magenta × green)*

F_3 PROGENIES FROM F_2 GREENS

Group no.	Pedigree no.	Year grown	Number of plants			Total number of plants
			Sun color	Questionable sun color	Green	
1	328-4-1	1928		9	5	14
	328-4-2	1928		14	1	15
	328-4-2	1929		48	1	49
	328-4-3	1928			3	3
	328-4-4	1928	1	10	3	14
	328-4-5	1928		7	3	10
	328-4-5	1929		57	2	59
	328-4-6	1928	1		1	2
	328-4-7	1928	26			26
	328-4-7	1929	1	29	10	40
	328-4-8	1928	7		2	9
	328-4-8	1929		10	1	11
	328-11-3	1929		13	6	19
2	327-2-1 to 7 inclusive	1929			115	115

F_4 PROGENIES FROM F_3 GREENS

3	328-4-1-3	1929		2	4	6
	328-4-1-8	1929		4	2	6
	328-4-4-10	1929			2	2
	328-4-5-1	1929			5	5
	328-4-8-1	1929		20	1	21
	328-4-8-2	1929		37	1	38
4	327-2-21-3	1929			25	25
5	327-2-21-6	1929			29	29
6	327-2-7-2	1929			14	14
	328-4-15-9	1929			14	14
	328-4-15-15	1929			9	9

F_4 PROGENIES FROM F_3 PLANTS RECORDED AS SUN COLOR

7	328-4-7-1	1929		5	5	10
	328-4-7-2	1929		1	2	3
	328-4-7-3	1929		[a] 3	7	10
	328-4-8-3	1929		27		27
	328-4-8-4	1929		12	1	13
	328-4-8-6	1929		12		12

F_4 PROGENIES FROM F_3 PLANTS RECORDED AS QUESTIONABLE SUN COLOR

8	328-4-1-1	1929		8	6	14
	328-4-1-6	1929		1		1
	328-4-1-10	1929		4	2	6
	328-4-4-4	1929			8	8
	328-4-5-2	1929		8	3	11
9	328-4-6-2	1929		[a] 8	1	9
	328-4-24-15	1929		32	6	38

[a] Plus 3 magenta in each progeny, probably the result of accidental cross-pollination.

The F_4 progeny from F_3 questionable-sun-color plants indicate that this type was not genotypically sun color, since no sun-color plants were produced.

Part of the F_3 green plants again produced plants of the questionable-sun-color type in varying proportions. Evidence presented in the section on intercrosses between F_2 color types indicates that the eight F_2 green plants and the F_3 questionable-sun-color and sun-color plants in their progeny were not genotypically sun color. Because of such evidence the questionable type plants have been classed with the green in all the progeny results to this point.

The appearance of green, questionable sun color, sun color, magenta, and purple in the same progeny discredits the possibility of multiple allelomorphs, and further tests will be necessary to determine whether the appearance of this questionable-sun-color type is due to environmental or modifying genetic factors.

INTERCROSSES OF F_2 COLOR TYPES

As a further check on the genetic constitution of the F_2 plants, crosses were made between the various color types found in this generation. The results are given in tables 3 and 4. Because of the labor involved in hybridization it was not possible to utilize all of the F_2 plants or to make all the possible or desired combinations. It will be noted that those crosses involving green are really back crosses. The genotypes assigned are those suggested by the F_2 or F_3 results.

TABLE 3.—*F_1 progenies of backcrosses and intercrosses between F_2 color types of crosses 328 (magenta × sun color) and 327 (magenta × green)*

Group no.	Families	Genotypes based on F_1 progeny						Number of plants				Total plants	Calculated ratio	P
		Purple	Magenta	Magenta	Sun color	Sun color	Green	Purple	Magenta	Sun color	Green			
	No.											*No.*		
1	1	[a] SsMm					ssmm	36	29	31	34	130	1:1:1:1	0.83
F_1 PROGENIES OF F_1 PURPLE OF CROSS 328 CROSSED WITH GREEN OF CROSS 327														
2	13	SsMm					ssmm	158	163	100	188	609	1:1:1:1	<0.01
3	5	SSMm					ssmm	60		68		128	1:1	.48
4	2	SsMM					ssmm	65	73			138	1:1	.50
5	3	SSMM					ssmm	109				109	All.	
F_1 PROGENIES OF CROSSES BETWEEN F_2 PURPLES AND SUN COLORS FROM CROSS 328														
6	1	SSMM			SSmm			50				50	All.	
7	3	SSMM			Ssmm			100				100	All.	
8	2	SSMm			SSmm			23		38		61	1:1	0.05
9	7	SSMm			Ssmm			16		14		30	1:1	.72
10	6	SsMm			SSmm			[b] 60		59		119	1:1	.99
11	19	SsMm			Ssmm			54	24	30	7	115	3:1:3:1	<.01

[a] Based on results of F_2 generation.

[b] Plus 2 magenta and 1 green, probably the result of accidental cross-pollination or self-pollination.

TABLE 3.—*F_1 progenies of backcrosses and intercrosses between F_2 color types of crosses 328 (magenta × sun color) and 327 (magenta × green)*—Continued

F_1 PROGENIES OF CROSSES BETWEEN F_2 PURPLES AND MAGENTAS FROM CROSS 328

Group no.	Families	Genotypes based on F_3 progeny						Number of plants				Total plants	Calculated ratio	P
		Purple	Magenta	Magenta	Sun color	Sun color	Green	Purple	Magenta	Sun color	Green			
	No.											*No.*		
12	1	SSMM	.....	ssMm	.....	.....	.....	23	.....	.....	.....	23	All.	.....
13	1	SSMm	.....	ssMM	.....	.....	.....	9	.....	.....	.....	9	All.	.....
14	3	SSMm	.....	ssMm	.....	.....	.....	20	.....	12	.....	32	3:1	0. 10
15	7	SsMm	.....	ssMM	.....	.....	.....	167	173	.....	.....	340	1:1	. 75
16	13	SsMm	.....	ssMm	.....	.....	.....	118	133	43	38	332	3:3:1:1	0. 10

F_1 PROGENIES OF MAGENTA × GREEN (CROSS 327)

17	1	.....	ssMM	.....	.....	.....	ssmm	.....	50	.....	.....	50	All.	.....

F_1 PROGENIES OF F_1 MAGENTA OF CROSS 327 CROSSED WITH GREEN OF CROSS 327

18	4	.....	ssMm	.....	.....	.....	ssmm	.....	79	.....	70	149	1:1	0. 47

F_1 PROGENIES OF F_2 MAGENTAS OF CROSS 327 CROSSED WITH GREEN OF CROSS 327

19	1	.....	ssMm	.....	.....	.....	ssmm	.....	14	.....	11	25	1:1	0. 56
20	3	.....	ssMM	.....	.....	.....	ssmm	.....	51	.....	.....	51	All.	.....

F_1 PROGENIES OF F_2 MAGENTAS OF CROSS 328 CROSSED WITH GREEN OF CROSS 327

21	4	.....	ssMm	.....	.....	.....	ssmm	.....	104	.....	100	204	1:1	0. 78
22	2	.....	ssMM	.....	.....	.....	ssmm	.....	52	.....	.....	52	All.	.....

F_1 PROGENIES OF CROSSES BETWEEN F_2 MAGENTAS AND SUN COLORS OF CROSS 328

23	1	.....	ssMM	.....	SSmm	.....	13	.....	.....	.....	.....	13	All.	.....
24	1	.....	ssMm	.....	SSmm	.....	20	.....	.....	32	.....	52	1:1	0. 10
25	5	.....	ssMM	.....	Ssmm	.....	106	.....	99	.....	.....	205	1:1	. 64
26	9	.....	ssMm	.....	Ssmm	.....	103	.....	98	53	68	322	1:1:1:1	<. 01

F_1 PROGENIES OF F_2 SUN COLOR OF CROSS 328 CROSSED WITH MAGENTA OF CROSS 327

27	4	.....	ssMM	.....	Ssmm	.....	30	.....	16	.....	.....	46	1:1	0. 04

F_1 PROGENIES OF CROSSES BETWEEN F_2 MAGENTAS OF CROSS 328

28	1	.....	ssMM	ssMM	.....	.....	.....	.....	57	.....	.....	57	All.	.....
29	2	.....	ssMm	ssMm	.....	.....	.....	.....	9	.....	6	15	3:1	0. 18

F_1 PROGENIES OF F_2 SUN COLORS OF CROSS 328 CROSSED WITH GREEN OF CROSS 327

30	5	.....	.....	.....	Ssmm	.....	ssmm	.....	.....	103	110	213	1:1	0. 65
31	3	.....	.....	.....	SSmm	.....	ssmm	.....	.....	106	.....	106	All.	.....

F_1 PROGENIES OF CROSSES BETWEEN F_2 SUN COLORS OF CROSS 328

32	4	.....	.....	.....	SSmm	Ssmm	.....	.....	.....	40	.....	40	All.	.....
33	3	.....	.....	.....	Ssmm	Ssmm	.....	.....	.....	46	6	52	3:1	0. 03

TABLE 4.—*F_1 progenies of crosses involving F_2 greens and questionable color types in F_3 from selfed F_2 green plants*

F_1 OF CROSSES BETWEEN MAGENTA AND F_2 GREENS

Group no.	Female parent	Male parent			Number of plants		
	Phenotype	Pedigree no.	Pedigree no.	Phenotype	Magenta	Questionable sun color	Green
1	Green	328-4-1	243-2-21	Magenta	15		
	do	328-4-2	do	do	6		
	do	328-4-4	do	do	9		
	do	328-4-5	do	do	2		
	do	328-4-6	do	do	27		
	do	328-4-7	do	do	28		
	do	328-4-8	do	do	13		
	do	328-11-3	do	do	12		

F_1 OF CROSSES BETWEEN MAGENTA AND F_3 TYPES FROM F_2 SELFED GREENS

2	Questionable sun color	328-4-1-1	243-2-21	Magenta	25		
	do	328-4-1-2	do	do	30		
	Green	328-4-1-3	do	do	21		
	Questionable sun color	328-4-1-4	do	do	13		
	do	328-4-1-6	do	do	18		
	Green	328-4-1-8	do	do	12		
	Questionable sun color	328-4-1-9	do	do	15		
	do	328-4-1-10	do	do	14		
	Green	328-4-4-1	do	do	8		
	do	328-4-4-2	do	do	14		
	Questionable sun color	328-4-4-4	do	do	18		
	do	328-4-4-5	do	do	17		
	do	328-4-4-6	do	do	6		
	do	328-4-4-7	do	do	17		
	do	328-4-4-8	do	do	18		
	do	328-4-4-9	do	do	17		
	Green	328-4-4-10	do	do	15		
	do	328-4-5-1	do	do	21		
	Questionable sun color	328-4-5-2	do	do	23		
	Sun color	328-4-6-2	do	do	20		
	do	328-4-7-1	do	do	15		
	do	328-4-7-2	do	do	3		
	do	328-4-7-3	do	do	14		
	Green	328-4-8-1	do	do	13		
	do	328-4-8-12	do	do	11		
	Sun color	328-4-8-3	do	do	7		
	do	328-4-8-4	do	do	3		
	do	328-4-8-6	do	do	8		
	Questionable sun color	328-4-24-15	do	do	13		

F_1 OF CROSSES BETWEEN F_2 GREENS FROM CROSS 328

3	Green	328-4-5	328-4-8	Green		8	
	do	328-4-5	328-4-7	do		1	
	do	328-4-5	328-4-6	do		1	
	do	328-4-6	328-4-8	do		1	3

F_1 OF CROSSES BETWEEN F_2 GREENS OF CROSS 328 AND GREEN OF CROSS 327

4	Green	328-4-1	243-2-5	Green			24
	do	328-4-2	243-2-5	do			22
	do	328-4-3	243-2-5	do			24

F_1 OF CROSSES BETWEEN F_2 GREENS OF CROSS 327 AND PARENTAL GREEN

5	Green	327-2-1	243-2-5	Green			23
	do	327-2-2	243-2-5	do			23

It is noteworthy that in every cross the expected color types were realized, and in the majority of cases the fit of observed to calculated frequencies of the involved color types was very good.

The deviations in group 2 of table 3 from the calculated 1:1:1:1 ratio is large ($P = < 0.01$), and practically precludes the possibility of the deviations being due to errors of random sampling. The deviations are greatest in the sun-color and green classes, there being a marked deficiency in the sun color and an excess in the green. An examination of the detailed records shows that more than 50 of the plants listed later as green were recorded originally as questionable sun color and were finally placed in the green class after the results of test crosses with these doubtful plants were known. In this connection it should also be stated that approximately half of the individuals in the larger families were classified on the basis of seedling plants, which may also account for a portion of the error. If, however, the sun-color and green classes are combined, P, calculated on the basis of a 1:1:2 ratio, becomes approximately 0.40. This good fit supports the idea that in this case the error was largely one concerned with classification.

In the case of groups 11 and 26, however, there was a deficiency in both the sun color and green classes. When the seedling cultures were grown in the greenhouse during the summer it was noticed that the purple and magenta plants were the most vigorous when all four types appeared in the same family. It is therefore possible that these smaller shaded plants were more subject to damping off and therefore to proportionately greater losses than the more vigorous purple or magenta plants. There may also have been some differential viability of the embryos.

In order to determine whether the F_2 plants classified as green were really green or light sun color as suggested by the F_3 data, they were crossed with magenta. If a mistake in classification had been made and the plants were really homozygous or heterozygous for the sun-color factor, the F_1 of a cross with magenta should be purple or approximately 50 percent purple, depending upon the genetic constitution of the sun-color plant. If it was green the F_1 should be uniformly magenta. The data in table 4, group 1, indicate that the eight F_2 plants tested were not sun color, since none of the back crosses contained any purple plants.

It will be recalled in the discussion of the F_3 results from selfed F_2 greens, that in addition to the expected green type some F_3 plants were sufficiently pigmented to apparently warrant being classified as sun color, others were called questionable sun color, and still others green. In order to check on the correctness of the classification, plants of all three types were crossed with the parental magenta.

The F_1 progeny results are given in table 4, group 2. None of the sun-color or questionable sun-color plants produced any purple plants in the F_1 and are therefore assumed not to be sun color. The pigmentation present was probably of a physiological nature due to the unfavorable growth conditions. These data corroborate the F_4 results obtained from the selfed F_3 plants, discussed in a previous section, which showed that none of the F_3 plants from F_2 greens produced sun color, magenta, or purple in the F_4.

The F_2 green plants were very poor producers of seed, and in all crosses where they were used as pistillate parents the number of progeny is accordingly small.

Four crosses were made between F_2 greens, and the F_1 progeny were either green or of the questionable sun-color type. Tests with similar questionable plants have shown (group 2 in table 4) that they were not genetically sun color.

Three of the F_2 green plants from cross 328 were back crossed to the 243–2–5 green, and all the 70 progeny were green, as shown in table 4, group 4.

Two green F_2 plants from the 327 cross were also crossed with the parental green and produced F_2 progenies of 46 green plants (table 4, group 5).

These results corroborate those of the F_3 and F_4 and furnish further proof of the correctness of the postulated genetic interrelations of the described color types.[6]

DISCUSSION

In attempting to interpret the results of previous work on the inheritance of cabbage color, in terms of the present report, one is confronted with the task of first solving the problem of color nomenclature. The absence of a standardized nomenclature makes it impossible to determine whether such terms as "red", "wine red", or "dark red violet" refer to the purple or magenta type as herein described. Pease placed "red" cabbage and "blue" kohlrabi under the common term "purple" which indicates their synonomy in Europe. It is also probable that sun color was not separated from pure green.

The clear-cut 3:1 segregations of red and green in the F_2 generation of crosses between red (purple) and green of Sutton, Pease, and Allgayer are probably best explained by assuming that the cross was made between purple and sun color, the classification being made on the basis of the color of the blade and neglecting the colored midrib of the sun-color type. It is also possible that the counts may have been made before the sun color developed sufficiently to become noticeable. Allgayer's description, however, agrees well with the writers' observations on the grades of color in an F_2 of a magenta × green cross, although it is possible that a summer record may reveal similar grades in the F_2 progeny of a sun color × purple cross.

Magenta crossed with green would also give a 3:1 segregation of colored to green. If the cross were made between purple and pure green it would be necessary to group purple and magenta together in the red class, and sun color and green in the green class to get a 3:1 ratio in the F_2 generation. It hardly seems possible that magenta should fail to be distinguished from purple, if present.

If it is assumed that Kristofferson's light-red-vein type is identical with the writers' sun color, and that the "dark red violet throughout" is the same as the writers' purple, then the results of his crosses of

[6] In a personal letter dated July 26, 1931, J. C. Miller, Louisiana State University, Baton Rouge, La., gives some results of a cross between an annual-seeding magenta from the junior author's cultures and an inbred line of the Jersey Wakefield variety which conforms to the sun-color type herein described. The F_1 plants were all purple and the F_2 contained approximately 9 purple: 3 magenta : 3 sun red : 1 green. F_2 data are not yet available. Miller found that the best time to detect the difference between sun red and green was during the months of December and January. His results corroborate those reported in the present paper.

light-red vein with green, with light-red vein and with dark-red violet throughout, agree with those in the present study.

If the green kale was really pure green, the results of the cross between it and dark-red violet cabbage do not agree with the writers' results from a purple × green cross. Kristofferson reports the F_2 as composed of approximately "3 dark red violet leaved plants to 1 green leaved plant." The veins of the green F_2 plants from the cross of dark-red violet × green kale were reported as being green. The same green kale crossed with light-red-veined cabbage produced an F_2 generation comprised of approximately 9 dark-red violet:3 light red:4 green vein, so it apparently brought in genetic factors not concerned in Kristofferson's above-mentioned results.

It is to be expected that other modifying factors or allelomorphs will be discovered as the above-described color types of *Brassica oleracea, var. capitata* are used in crosses with other varieties of this species or with other strains of this botanical variety.

SUMMARY

This paper reports the results of inheritance studies with purple, magenta, sun color, and green plant colors of cabbage, *Brassica oleracea*, var. *capitata*.

Data are presented on crosses between magenta with green and sun color, back crosses of the F_1 and F_2 color types with the double recessive green, behavior of F_2 color types in the F_3 and F_4 generations, and progenies of intercrosses between F_2 color types.

The results are interpreted as supporting the following genetic formulas for the respective color phenotypes used in this study:

SM = purple	sM = magenta
Sm = sun color	sm = green

The existence of all possible genotypes of these phenotypes has been demonstrated in the course of this work.

LITERATURE CITED

(1) Allgayer, H.
1928. Genetische untersuchungen mit gartenkohl (brassica oleracea) nach kreuzungsversuchen von richard freudenberg. Ztschr. Induktive Abstam. u. Vererbungslehre 47: [191]–260, illus.

(2) Fisher, R. A.
1930. Statistical methods for research workers. Ed. 3, rev. and enl., 283 p., illus. Edinburgh and London.

(3) Kristofferson, K. B.
1924–27. Contributions to the genetics of brassica oleracea. (I-II). Hereditas 5: [297]–364, illus., 1924; 9: [343]–348, illus., 1927.

(4) Moldenhawer, C.
1927. Recherches sur certains croisements interessants du genre brassica. Bul. Internatl. Acad. Polon. Sci. et Lettres, Cl. Sci. Math. Nat. (B) 1927: 1049–1071, illus.

(5) Pease, M. S.
1926. Genetic studies in brassica oleracea. Jour. Genetics 15: [363]–385, illus.

(6) ———
1927. Genetic studies in brassica oleracea. II. the kohl rabi. Jour. Genetics 17: [253]–267, illus.

(7) SUTTON, A. W.
1908. BRASSICA CROSSES. Jour. Linn. Soc. [London], Bot. 38: 337–349, illus.
(8) SUTTON, E. P. F.
1924. INHERITANCE OF "BOLTING" IN CABBAGE. Jour. Heredity 15: 257–260, illus.
(9) WIEGMANN, A. F.
1828. ÜBER DIE BASTARDERZEUGUNG IM PFLANZENREICHE. 40 p., illus. Braun schweig. (K. Akad. Wiss. Berlin Gekrönte Preisschrift) [Original not seen.]

THE COMPOSITION OF FEEDSTUFFS IN RELATION TO NUTRITIONAL ANEMIA IN CATTLE[1]

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INTRODUCTION

Cattle suffer from a nutritional anemia, locally called "salt sick," on certain definite areas of the coastal plains. Affected animals recover when moved to certain other ranges but become salt sick again within a few months if returned to the original range. Cattle are moved from range to range in this manner to enable the owners to utilize the pastures and to keep their cattle healthy.

In cooperative work with owners of affected cattle (*7*)[3], this nutritional anemia was corrected by feeding supplements of iron, or of iron and copper, without other changes in feeding or management, thus enabling owners to make maximum use of affected lands without shifting their cattle.

It was noted from a field survey (*7*) that the affected ranges consisted of white and gray sands, cumulose muck lands, and shallow residual soils overlying marl. Healthy ranges were on red and yellow sands, clays, shallow sands underlaid with clay, and soils subject to overflow from clay lands.

Forage and grain samples were collected at the time of the cooperative work and field survey in order to determine quantitative differences in the composition of such samples from the various areas. Analyses of the feedstuffs are reported in this paper.

LITERATURE REVIEW

A deficiency of iron has been found in the natural rations of livestock in several parts of the world. Aston (*3*, *4*) demonstrated this deficiency to be the cause of the condition known as "bush sickness," or "skinnies" in sheep and cattle in parts of the counties of Rotorua, Matamata, and Tauranga in the North Island, New Zealand. Orr (*20*) and Gunn (*12*) have shown that "pining" among the sheep in the Cheviot Hills in the south of Scotland, and "nakuruitis" in the Nakura district of Kenya Colony are both associated with this deficiency. Dickinson (*9*) has verified Aston's prediction that "coasty disease" on King Island, Tasmania, is of the same etiology. The condition found in Florida (*7*, *17*, *19*) is complicated in certain areas by a concurrent deficiency of copper.

Aston and his associates (*5*, *6*), and Askew and Riggs (*1*) made comparative analyses of forages from affected and healthy areas in New Zealand and found definite differences in composition of the crops from the two areas, which will be discussed later in relation to the data presented.

[1] Received for publication Jan. 26, 1933; issued August 1933.
[2] The assistance of J. R. Greenman and William T. Dunn with parts of the analytical work herein presented is acknowledged.
[3] Reference is made by number (italic) to Literature Cited, p. 254.

The evidence of iron deficiency in other areas consists of soil analyses from the healthy and affected areas, and the prevention and correction of the condition in sheep and cattle by administration of iron compounds.

METHODS OF INVESTIGATION

As soon as salt sick in cattle had been demonstrated to be a nutritional anemia caused by a lack of iron, or of iron and copper (*7*, *17*), the collection of a series of feed samples from healthy and affected areas was instituted. Designation of such areas was made according to statements of cattle owners based on their experience, and upon direct observations of cattle on these areas. In some few places, the deficiency was so extreme that the landholders had abandoned any further attempt to handle cattle. In many other places, few attempts were made to raise young stock.

Wire grass (*Aristida* species, mostly *A. stricta*) was selected as the forage to indicate whether or not the degree of deficiency on the various ranges, and the relation of stage of growth and season of the year to the incidence of the condition, could be measured analytically. This particular grass was selected because of its predominance on the majority of the range areas. Other forages were collected incidental to the investigation.

Samples gathered to show the effect of stage of growth were always taken from unfertilized land and on sites not subject to overflow during the rainy season. Successive samples were taken from identical areas. Whenever possible, adjacent samples were taken on burned and unburned range. This was done because of the practice of "burning the woods" (*8*), by turpentine operators to protect their cups from uncontrolled or accidental burning, and by cattlemen to provide succulent new growth in the late winter and early spring resulting from burning off the "rough." Wire grass was gathered by pulling the individual blades. Only living grass was taken, representative of the growth on the land.

Corn was selected to represent the grains, as it is the only grain grown extensively in Florida. Velvetbean samples were secured whenever possible. This latter crop generally is harvested by pasturing in the fall and winter, when the grasses are short or old. Some cattlemen believe that it aids in a natural recovery from nutritional anemia. This belief may be due to the fact that the velvetbeans usually are grown on the more fertile or fertilized lands, thus having a higher content of the mineral elements.

Particular care was exercised to avoid soil contamination, as only a small amount of clay soil adhering to the plants would lead to a large error in an iron analysis. This error is greatest in the case of low-growing forages such as lespedeza, and ensiled crops where handling in the field is unavoidable. After drying, the samples were ground in a clean Wiley mill. Any iron contamination from this source should be nearly uniform for all samples.[4]

Analyses made included calcium, magnesium, and phosphorus by the method of Morris, Nelson, and Palmer (*16*), iron by a modification of the method of Kennedy (*15*), and proximate analyses of some of the crops more or less peculiar to Florida by the methods of the Association of Official Agricultural Chemists (*2*).

[4] See footnote on opposite page.

RESULTS

Gradation was observed as to the quality of the various ranges. On some it is impossible to maintain cattle; on others the condition of salt sick never occurs, while on the marginal areas cattle do not develop salt sick, nor do affected cattle placed on them make recovery. Forage samples from marginal ranges have been omitted from this study.

Wire grass is the principal forage on the open ranges other than the muck lands of this region. It makes some growth during the entire year, but the proportion of older growth and dead grass increases progressively. During the winter this is sufficient to interfere with easy grazing, and hence parts of the range are burned over periodically. Wire grass, being a bunch grass, withstands this burning and immediately puts out a succulent new growth.

The comparison of the mineral content of samples of wire grass from both healthy and affected areas that have been burned over, or left rough, is presented in table 1, together with a comparison with other feed crops.

The wire-grass samples from the healthy range areas show a uniformly higher content of all the minerals determined than do those from the affected areas, in general amounting to approximately 20 percent. The difference between burned and unburned areas is greatest in the early spring and almost disappears by July. The seasonal trend on both burned and unburned areas is toward a decreased mineral content, a late sample from a healthy area possibly showing a lower mineral content than does an early sample from an affected area.

Other forages produced on affected lands had a greater percentage of all the mineral elements except iron than did the wire grass. The legumes were very much higher in calcium and phosphorus. The significantly higher percentages of iron in lespedeza and in soybean-silage samples may be attributed in part to soil contamination. The lespedeza was of a low decumbent growth. Slight soil contamination was visible in the silage samples. Differences due to soil contamination would tend to magnify themselves between healthy and affected soils because of the higher iron content of the healthy soils associated with the increase in the clay fraction.

[4] Since this manuscript was prepared, the following analyses have been made to determine the extent of iron contamination from the Wiley mill. There is some increase in the iron content due to grinding in the Wiley mill. The differences between areas reported, considering that averages of a number of samples have been given, remain significant.

Description of sample	Iron content of sample	
	Before grinding	After grinding
Wire grass:	*Percent*	*Percent*
Sample A	0.0194	0.0222
Sample B	.0213	.0197
Sample C	.0199	.0288
Sample D	.0174	.0206
Sample E	.0213	.0230
Average	.0196	.0228
Velvetbean seed	.0105	.0146
Velvetbean pods	.0084	.0161
Corn, grain	.0083	.0172

TABLE 1.—*Mineral content of the dry matter of various feedstuffs in relation to nutritional anemia in cattle*

FORAGES

Feedstuff and month cut	Samples from healthy areas					Samples from salt-sick areas				
	Samples	Ca	Mg	P	Fe	Samples	Ca	Mg	P	Fe
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
Wire grass (burned area):										
March	6	0.230	0.178	0.276	0.0256	7	0.153	0.132	0.221	0.0191
April	6	.221	.167	.231	.0233	7	.177	.151	.208	.0184
May	6	.181	.122	.169	.0240	7	.140	.100	.144	.0194
June	6	.167	.110	.122	.0207	7	.129	.090	.117	.0172
July	6	.142	.095	.106	.0172	7	.117	.078	.106	.0143
Total or average	30	.188	.134	.181	.0222	35	.143	.110	.159	.0177
Wire grass (unburned area):										
March	2	.262	.096	.209	.0196	2	.153	.117	.108	.0165
April	2	.193	.107	.168	.0217	2	.167	.148	.164	.0182
May	2	.190	.108	.171	.0209	2	.114	.086	.102	.0155
June	2	.200	.077	.125	.0177	2	.107	.086	.102	.0218
July	2	.203	.086	.118	.0164	2	.107	.074	.096	.0120
Total or average	10	.210	.095	.158	.0193	10	.127	.102	.114	.0168
Alfalfa hay, no. 1[1]	9	1.173	.164	.251	.0214					
Beggarweed	2	1.158	.272	.293	.0181					
Corn silage						9	.274	.179	.291	.0191
Lespedeza	6	1.086	.227	.240	.0617	2	1.114	.479	.286	.0165
Natal grass	1	.508	.247	.330	.0385	2	.489	.313	.310	.0130
Soybeans	15	1.083	.365	.406	.0255					
Soybean silage	23	1.178	.369	.406	.0402					

GRAINS AND BY-PRODUCTS

Cornmeal	6	0.016	0.122	0.291	0.0128	9	0.011	0.120	0.332	0.0094
Velvetbean seed	6	.160	.184	.451	.0110	3	.138	.180	.421	.0102
Velvetbean feed meal	6	.291	.205	.335	.0113	3	.243	.206	.322	.0113
Cottonseed meal, 36 percent protein	2	.235	.975	.362	.0196					
Cottonseed meal, 43 percent protein	3	.251	1.001	.375	.0134					

[1] Purchased on the Kansas City market in 1929, 1930, and 1931.

Corn meal (from shelled grain) showed a higher iron content from the healthy areas, while no difference was evident between the velvetbean samples analyzed. Cottonseed meal milled in Alabama and Georgia from seed grown on red clay soils had a higher iron content than did either the corn or velvetbeans. The 36 percent protein grade of meal with its higher proportion of hulls had a higher iron content than had the 43 percent meal. Cottonseed meal is considered one of the curative feeds. Proximate analyses of some of the above samples are presented in table 2.

The higher crude-protein content of the wire grass from burned ranges, and the remarkably constant percentage of crude fiber regardless of stage of growth of this plant are particularly significant. It would seem to be a desirable forage in the earlier stages of growth, as has been proven by experience. The carbohydrate content would seem to be adequate at any season.

The lespedeza samples were collected during the bloom stage, when the growth was desirable for pasturage. The soybean silage was made from soybeans in an earlier stage of growth than those reported elsewhere (*18*). The protein content of the velvetbeans was higher than in those summarized by Henry and Morrison (*14*).

TABLE 2.—*Proximate analyses of some of the feedstuffs listed in table 1*

Feedstuff and month cut	Samples	Moisture	On moisture-free basis				
			Ash	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract
Wire grass (burned area):	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
March	7	----------	5.08	10.70	34.01	48.79	1.41
April	7	----------	5.24	9.26	34.40	49.76	1.34
May	7	----------	3.92	6.52	35.03	52.98	1.55
June	7	----------	3.12	4.46	36.21	54.69	1.52
July	7	----------	3.23	3.60	36.90	54.63	1.64
Wire grass (unburned area):							
March	3	----------	3.60	4.26	33.35	56.99	1.80
April	3	----------	3.56	4.64	34.72	55.41	1.67
May	3	----------	3.25	4.49	35.00	55.59	1.67
June	3	----------	2.64	3.90	36.83	55.07	1.56
July	3	----------	2.81	3.73	37.40	54.53	1.53
Beggarweed	2	8.98	4.89	15.78	36.88	40.23	2.23
Lespedeza	7	9.66	5.86	14.93	26.33	50.71	2.17
Soybeans, green	15	74.91	9.34	12.00	36.32	39.74	2.55
Soybean silage	23	75.43	10.70	9.04	40.51	36.54	3.20
Velvetbean seed	9	11.08	3.25	27.07	7.29	56.64	5.10
Velvetbean feed meal	9	11.35	3.66	20.95	13.79	57.30	4.30

DISCUSSION

Differences in mineral contents of wire grass from burned and unburned areas were greater than those observed by Hart, Guilbert, and Goss (*13*) in alfilarai and wild oats. Grazing animals preferred the forage on the burned range, as Camp (*8*) also has pointed out. The analyses herein presented explain to some extent why cattle should make better growth and be more thrifty on such ranges. However, this report makes no attempt to evaluate the practice of burning the range, either for a single season or over a number of years.

The new grass on a burned area was of a more recent growth and had a higher mineral and protein content than had grass taken from adjacent unburned areas. This difference incident to stage of growth and season of the year, has been observed with other pasture plants (*1, 6, 10, 13, 20, 21*). Most of the samples included in these reports represent the new growth after clipping, whereas the Florida samples represent the entire accumulation of growth before the time of sampling, except as reduced by incidental grazing. The results reported are therefore more nearly due to stage of growth than to the season of the year.

The fact that the poverty of iron, rather than any of the other minerals that parallel it, was the factor limiting the nutritive value of the wire grass, was proven in cooperative feeding trials with cattle on affected ranges (*7, 19*). A supplement of bonemeal, without any change in feeding practice, did not lead to improvement in condition of salt-sick cattle on affected wire-grass ranges, while the administration of an iron-and-copper supplement was effective. The use of other forages grown on affected soils, with their higher content of the mineral elements other than iron, did not alleviate the condition.

Whether or not animals can utilize the iron contained in soil contamination (of the forages) is unknown. If they can, then the difference between the iron intake of cattle on the two types of range would be greater than is indicated by analyses of the feedstuffs. Askew and Riggs (*1*) have offered this explanation to account for finding an iron content of 0.008 percent from bush-sick range forage,

and of 0.009 and 0.011 percent from healthy range forages at Glenhope, New Zealand. These percentages are lower than those presented herein. However, all New Zealand analyses were discarded if there were any indications, whatever, of soil contamination. They represent season averages.

Aston (*3*, *4*) gave the iron percentages on plant ash from healthy and affected areas. The differences observed were verified in a later report (*5*) which included the percentage of iron in the dry matter. Red clover, white clover, and crowfoot grass had an iron content in the dry matter of 0.0077, 0.0084, and 0.0098 percent, respectively, when grown on bush-sick soils, and 0.0147, 0.0119, and 0.0119 percent respectively, when grown on healthy soils. Samples from marginal areas had an iron content between these values.

Godden and Grimmett (*11*) found differences in the amount of iron in the dry matter of oats and mustard grown in pot cultures, using soils from pining and bush-sick lands, in comparison with healthy soils.

The differences observed between the forages of healthy and affected areas in the various parts of the world are comparable, even though the absolute concentrations vary. This may be due to differences in methods of analysis. All the evidence reported supports the results secured by the feeding of supplements to the livestock in the regions under consideration.

SUMMARY AND CONCLUSIONS

The percentage of calcium, magnesium, phosphorus, and iron tend to decrease with advancing stage of growth in wire grass, this tendency being more marked on the burned than on the unburned range areas in Florida. A similar trend is noted in the crude protein content of this grass. These differences are associated with stage of growth, and practically disappear by midsummer.

Wire grass grown on salt-sick range areas contained less iron, calcium, magnesium, and phosphorus than did that from areas where cattle are not affected with this nutritional anemia. Other forages and grains analyzed also followed this trend.

These differences support the results obtained by use of iron-containing supplements, with livestock on ranges where the condition called salt sick occurs, and are in harmony with investigations in other known iron-deficient regions.

LITERATURE CITED

(1) Askew, H. O., Riggs, T., Grange, L. I., and Taylor, N. H.
1932. bush sickness. investigations concerning the occurrence and cause of bush sickness in new zealand. New Zeal. Dept. Sci. and Indus. Research Bul. 32, 62 p., illus.

(2) Association of Official Agricultural Chemists.
1925. offical method of analyses. compiled by the committee on editing methods of analyses. revised to july 1, 1924. Ed. 2, 535 p., illus. Washington, D.C.

(3) Aston, B. C.
1924. bush-sickness investigation. five years' work at the mamaku demonstration farms. New Zeal. Jour. Agr. 28: [215]–238, illus.

(4) ———
1924. the bush-sickness investigation. laboratory work and results. New Zeal. Jour. Agr. 28: 301–305, 381–390, illus.; 29: 84–91.

(5) ASTON, B. C.
1928. MINERAL CONTENT OF PASTURES. PROGRESS OF THE NEW ZEALAND INVESTIGATION. New Zeal. Jour. Agr. 36: 22–27, [75]–82.
(6) ——— GRIMMETT, R. E. R., BROGAN, F. J. A., and SYKES, P. H.
1931. MINERAL CONTENT OF PASTURES. THE WAIRARAPA DISTRICT. New Zeal. Jour. Sci. and Technol. 12: 304–320.
(7) BECKER, R. B., NEAL, W. M., and SHEALY, A. L.
1931. I. SALT SICK: ITS CAUSE AND PREVENTION. II. MINERAL SUPPLEMENTS FOR CATTLE. Fla. Agr. Expt. Sta. Bul. 231, 23 p., illus.
(8) CAMP, P. D.
1932. A STUDY OF RANGE CATTLE MANAGEMENT IN ALACHUA COUNTY, FLORIDA. Fla. Agr. Expt. Sta. Bul. 248, 28 p., illus.
(9) DICKINSON, C. G.
1927. KING ISLAND "COASTY" DISEASE. Aust. Vet. Jour. 3: 82–85.
(10) ELLIOTT, W., ORR, J. B., WOOD, T. B., and CRUICKSHANK, E. M.
1926. INVESTIGATIONS OF THE MINERAL CONTENT OF PASTURE GRASS AND ITS EFFECT ON HERBIVORA. IV. REPORT ON THE SEASONAL VARIATIONS IN THE MINERAL CONTENT OF PASTURE. Jour. Agr. Sci. [England] 16: 89–97, illus.
(11) GODDEN, W., and GRIMMETT, R. E. R.
1928. FACTORS AFFECTING THE IRON AND MANGANESE CONTENT OF PLANTS, WITH SPECIAL REFERENCE TO HERBAGE CAUSING "PINING" AND "BUSH-SICKNESS." Jour. Agr. Sci. [England] 18: [363]–368.
(12) GUNN, H.
1928. PASTURE INVESTIGATION IN KENYA. NOTES ON "NAKURUITIS." Kenya Colony Dept. Agr. Bul. 24, 2 p.
(13) HART, G. H., GUILBERT, H. R., and GOSS, H.
1932. SEASONAL CHANGES IN THE CHEMICAL COMPOSITION OF RANGE FORAGE AND THEIR RELATION TO NUTRITION OF ANIMALS. Calif. Agr. Expt. Sta. Bul. 543, 62 p., illus.
(14) HENRY, W. A., and MORRISON, F. B.
1923. FEEDS AND FEEDING; A HANDBOOK FOR THE STUDENT AND STOCKMAN. Ed. 18, 770 p., illus. Madison, Wis.
(15) KENNEDY, R. P.
1927. THE QUANTITATIVE DETERMINATION OF IRON IN TISSUES. Jour. Biol. Chem. 74: 385–391, illus.
(16) MORRIS, H. P., NELSON, J. W., and PALMER, L. S.
1931. QUANTITATIVE DETERMINATION OF CALCIUM, MAGNESIUM, AND PHOSPHORUS IN FEEDSTUFFS AND CATTLE EXCRETA. Indus. and Engin. Chem., Analyt. Ed. 3: 164–167.
(17) NEAL, W. M., and BECKER, R. B.
1933. THE HEMOGLOBIN CONTENT OF THE BLOOD OF HEALTHY AND ANEMIC "SALT-SICK" CATTLE. Jour. Agr. Research 46: 557–563.
(18) ——— and BECKER, R. B.
1932. A CHEMICAL STUDY OF ENSILING SOYBEANS. Jour. Agr. Research 46: 669–673.
(19) ——— BECKER, R. B., and SHEALY, A. L.
1931. A NATURAL COPPER DEFICIENCY IN CATTLE RATIONS. Science (n.s.) 74: 418–419.
(20) ORR, J. B., and HOLM, A.
1931. THE INFLUENCE ON ANIMAL HEALTH OF MINERALS IN DIET. THE MINERAL DEFICIENCIES OF PASTURES IN KENYA COLONY AND THEIR EFFECTS ON GRAZING ANIMALS. [Gt. Brit.] Econ. Advisory Council Com. on Mineral Content of Natural Pastures, Rpt. 6: 12–66, illus.
(21) ——— and SCHERBATOFF, H.
1929. MINERALS IN PASTURE AND THEIR RELATION TO ANIMAL NUTRITION. 150 p., illus. London.

U. S. GOVERNMENT PRINTING OFFICE: 1933